AUTOCRINE GROWTH REGULATION OF
A CLONED MURINE T HELPER CELL LINE

JAY BRUCE HOROWITZ

1987

Autocrine Growth Regulation of a Cloned Murine T Helper Cell Line

A Thesis Submitted to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine

by

Jay Bruce Horowitz

1987

ABSTRACT

Autocrine Growth Regulation of a Cloned
Murine T Helper Cell Line

Jay Bruce Horowitz

Yale University School of Medicine

1987

The growth and proliferation of mammalian cells are controlled in part by polypeptide hormones found in the serum. Malignant or transformed cells appear to require no or lower concentrations of growth-controlling factor(s) as compared with normal or untransformed cells. It has therefore been suggested that cells could become malignant via the aberrant production of polypeptide growth-controlling factor(s) acting in an autocrine manner through functional cell membrane receptors resulting in uncontrolled proliferation. Recently, the "autocrine hypothesis" has been further defined in that malignant transformation may be the result not only from excessive production, expression, and action of positive autocrine growth factors but also the failure of cells to synthesize, express or respond to specific negative growth factors that they normally release to control their own growth.

It had been shown that the T helper cell line D10 secretes large amounts of a T cell growth factor, believed to be IL-2, upon crosslinking of its antigen-class II MHC products receptor yet failed to proliferate. However, if the monokine IL-1 was present during stimulation potent proliferation occurred. Furthermore, D10 cells have to been shown to both respond to IL-2 derived from a T cell hybridoma and to express functional IL-2 receptors. Thus in the presence of both functional T cell growth factor and IL-2 receptors this clone fails to proliferate.

It appears that there are at least two possible explanations of why D10 fails to proliferate in response to crosslinking of its antigen-class II MHC products receptor. The first explanation is that D10 does not secrete IL-2 but IL-4. D10 cells do not respond vigorously to purified IL-4, probably because the cells do not express significant numbers of IL-4 receptors. However, if IL-1 and IL-4 are incubated together with D10 cells, the cells are able to proliferate in response to IL-4. Secondly, in addition to IL-4, D10 produces an inhibitor of T cell responses to IL-2 and possibly IL-4. Although the relative contribution each plays has yet to be definitively determined, the initial evidence suggests that the IL-1 dependence of D10's autocrine growth is largely if not entirely due to the requirement for IL-1 as a cofactor for IL-4 driven T cell growth. The precise mechanisms of this IL-1 dependence is currently being studied.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

INTRODUCTION

HISTORICAL PERSPECTIVE

Although many of the advances in immunology have occurred in the past twenty years, references to the immune system and immunology can be found dating back to the bible and before. Thucydides, in his description of the plague that struck Athens in 430 B. C. noted: "Yet it was with those who had recovered from the disease that the sick and the dying found most compassion. These knew what it was from experience, and had now no fear for themselves; for the same man was never attacked twice--never at least fatally."[1] In describing the plague of the Byzantine emperor Justinian a thousand years later, Procopius said "at a later time it [the plague] came back; then those who dwelt round about this land, whom formerly it had afflicted most sorely, it did not touch at all."[1] In time this resistance to reinfection become known as immunity, from the Latin *immunitas* [2].

It was not until the late 19th century when the importance of cells was finally appreciated that immunology became a true science. Before that time, medicine had been under the domination of the old Greek view that disease was based on imbalances in the "essential bodily humors." Metchnikoff, based on his observations of phagocytic cells in marine invertebrates, suggested that vertebrate phagocytic cells performed a similar protective function[3]. Shortly after the initial description of the phagocytic theory, it came under severe and protracted attack with the fiercest criticism coming from Baumgarten[3]. At about the same time, Nuttall noted that the serum of normal animals contained substances which were toxic to certain microorganisms, eg. bacteria, and whose properties were enhanced when the host was immunized[1]. In 1890 von Behring and Kitasato described humoral immunity to diphtheria and tetanus[1]. With this and other published reports, the humoral theory of immunology gained

converts while supporters of the cellular theory dwindled. It was not until Medawar's observations on tissue transplantation and immunologic tolerance, Burnet's emphasis on the importance of cells in antibody production, and descriptions on the role of the thymus in immunologic deficiency states in the 1950s and 1960s that a renewed interest cellular immunology finally occurred[1].

BRIEF OVERVIEW

The immune system has classically been divided into cell mediated and humoral components. Humoral immunity is provided by antibodies that circulate in the serum and in the lymphatic fluid. Cellular immunity, as its name implies, is provided by specific cells in the immune system. Although there is much overlap between the two "arms," for descriptive purposes it is still relevant to talk in these terms. People with deficiencies in humoral immunity are plagued with recurrent or chronic sinopulmonary infections, meningitis, and bacteremias caused by pyogenic bacteria while abnormalities in cell mediated immunity predisposes individuals to disseminated viral and fungal as well as protozoal infections[4].

The constituents of the immune system include lymphocytes, macrophages and their related cells, eg. dendritic cells of the spleen, Kupffer cells of the liver, epithelial Langerhans cells, etc., and specialized epithelial cells, such as those found in the thymus[5]. These cells may be found in organized tissues and organs, such as the lymph nodes, the spleen, the tonsils, the Peyer's patches of the intestine, the thymus, and the bone marrow or may circulate throughout the body in the blood and in the lymphatic fluid[5].

There are two major classes of lymphocytes: B lymphocytes which are the precursors of antibody secreting plasma cells and T lymphocytes which are thymus dependent and mediate important regulatory functions including augmentation and suppression of the immune response and antibody

production[5]. Both B lymphocytes and T lymphocytes are derived from hematopoietic stem cells located within the bone marrow and in other hematopoietic tissue. Mononuclear phagocytes are the third type of cell that is intimately involved in the development and expression of humoral and cell-mediated immunity[6]. These cells participate in a number of events in the immune response including host defense against microorganisms, cellular interactions with lymphocytes, and secretion of monokines[6].

ANTIGEN PRESENTING CELLS

The first step that occurs when a foreign material (antigen) enters the body is its uptake by cells of the phagocytic system, usually by nonimmunological means[6]. Antigen is then processed by the cell such that an antigen fragment[7] or native antigen[8] is present on the surface of the antigen presenting cell (APC). Antigen in association with self class II major histocompatibility complex (MHC) products is then presented to the T cell receptor complex[9]. This in turn, leads to T cell activation, proliferation[10] and production of various T cell lymphokines, primarily T cell growth factor (TCGF)[11].

Currently, there is much controversy over which cell type or types present antigen *in vivo*. A number of different cell types have been reported in the literature: peritoneal and splenic macrophages[12], splenic dendritic cells[13], epidermal Langerhan cells[14], Kupffer cells[15], alveolar macrophages[16], B cells[17], and B cell tumors[18]. Each, under the appropriate experimental conditions, are able to act as APCs. The biological significance of all these different cell types acting as APCs remains unknown.

T LYMPHOCYTES

OVERVIEW

T lymphocytes (or T cells), so called because their maturation requires processing in the thymus[19], are the central cells in the cellular immune response. They generate cytotoxic responses to alloantigens, exert helper and suppressor effects on the production of antibodies, and initiate graft versus host responses[20]. Cantor and Boyse, in a series of elegant experiments, demonstrated that commitment of T cells to participate exclusively in either helper or cytotoxic function is a differentiative process that takes place before they encounter antigen[20]. They further showed that T cells involved in helper responses expressed different surface antigens (Ly-1$^+$2$^-$) than those involved in killing allogeneic target cells (Ly-1$^-$2$^+$)[20]. Cantor and Boyse also noted that Ly-1$^+$2$^-$ T cells recognized self class II MHC products while Ly-1$^-$2$^+$ T cells recognized self class I MHC products[21]. Rao et al. confirmed the initial observation of Cantor and Boyse by using 21 T cell clones derived from mixed lymphocyte cultures and studying the association of Ly phenotype with function and specificity for MHC products[22].

As mentioned above, antigen together with class II MHC products are presented by APCs to T helper cells. This in turn results in proliferation and elaboration of a variety of lymphokines. The T helper cell binds the antigen-self class II MHC complex through its receptor, a hetrodimer composed of a disulfide linked acidic (α) and basic (β) chains[23]. Although the antigen-self class II MHC complex is the apparent ligand for the T cell receptor, it appears that receptor cross linking or aggregation is the necessary requirement for T cell activation[24]. In addition to receptor cross linking it has been shown that certain T cells require interleukin 1 (IL-1) for the secretion of T cell lymphokines, mainly

interleukin 2 (IL-2)[25,26]; while, in other T cells IL-1 was required for the expression of IL-2 receptors[27]. However Malek et al., using a heterogeneous population of resting T helper cells essentially depleted of accessory cells, found that IL-1 neither induced IL-2 secretion nor promoted IL-2 receptor expression[28].

ACTIVATION

In addition to activation via antigen and self class II MHC products, T helper cells may be activated with plant lectins such as phytohemagglutinin (PHA) and concanavalin A (Con A)[29], calcium ionophores such as A23187[30], and monoclonal antibodies[31,32,33,34].

The term lectin refers to a large collection of carbohydrate binding multimeric glycoproteins derived from plant and animal sources[35]. There are at least several hundred plant extracts that interact with and agglutinate erythrocytes; however, only a small number bind to lymphocytes and induce a mitogenic response[36]. Of these PHA from the red kidney bean (*Phaseola vulgaris*) and Con A from the jack bean (*Canavalia ensiformis*) are the most widely used T cell lectins[36]. Although the mechanism by which lectins interact with cell surface proteins remains largely unknown, they apparently work by cross-linking cell surface molecules[29]. In a recent study, Tsien et al. have partially unraveled the mechanism of lectin induced activation with their finding of increased intracellular calcium following lectin binding[37].

Calcium ionophores, such as A23187, work by directly inserting into the plasma membrane forming a calcium ion channel which results in a rapid influx of calcium[38]. Akerman and Andersson, using purified human T cells, showed polyclonal proliferation following treatment with A23187[30]. This finding combined with those of Tsien et al. suggest that an increase in cytosolic calcium directly triggers the proliferative response.

Monoclonal antibodies directed at the T cell receptor complex have allowed further definition of the mechanism involved in activation of T cells[31-34]. Kaye et al. described a monoclonal antibody (3D3) specific for the cloned helper T cell line D10.G4.1 (D10) that completely replaced the requirement for antigen and self class II MHC products in the activation of that clone[24,34]. Using the Fab fragment from that monoclonal antibody as an inhibitor of activation, activation of D10 by either antigen-self class II MHC products or allogeneic class II MHC products was blocked. The ability of the Fab fragment to induce proliferation was restored when anti-mouse immunoglobulin was used to cross link the Fab fragments. They also noted that the ability of 3D3 to activate D10 was dependent on a signal from an accessory cell and that by using either IL-1 or IL-2 the requirement for accessory cells could eliminated. (Note: The source of IL-2 used in those experiments has recently been shown to contain an additional TCGF, see results). The conclusions to be drawn from these experiments are (1) the T cell receptor complex is the binding site for both antigen-self class II MHC products and allogeneic-class II MHC products, (2) receptor cross linking and not receptor occupancy provides the necessary signal for activation , and (3) activation for T cell growth is a two signal process.

CLASSIFICATION OF T HELPER CELLS

Murine T helper cells appear to be fairly uniform when viewed in the context of surface markers such as Ly-1; however, when assessed by functional criteria at least two[39,40,41,42] and as many as four[43] different types of T helper cells have been described. Mosmann et al. screening a large panel of antigen-specific murine T helper cell clones found that two types of functionally distinct cells could be distinguished based on lymphokine secretion[44]. Mosmann Type 1 T helper cells when stimulated with antigen and APCs or Con A produced IL-2, interferon-γ (IFN-γ), granulocyte-macrophage colony stimulating factor (GM-

CSF), and interleukin 3 (IL-3) while Mosmann Type 2 cells secreted IL-3 and interleukin 4 (IL-4). Kim et al.; however, divided Ly1+2- cells into four functionally distinct types based upon their ability to induce a phosphorylcholine-specific (PC) plaque-forming cell response, their requirement for the presence of antigen during induction of B cell responses, and their activation of T15-bearing or non-T15-bearing B cells[43]. (T15 is the prototypic idiotypic determinant characteristic of the BALB/c PC-binding myeloma protein TEPC-15)[45]. Types 1 and 2 induce B cells to secrete anti-PC antibodies in an antigen specific class II-MHC products restricted fashion. Type 3 cells induce antigen-specific class II-MHC products restricted B cell proliferation, but do not lead to specific antibody formation, and Type 4 cells are autoreactive and induce antigen-independent B cell activation and antibody secretion. Killar et al. have recently confirmed the finding that IL-4 producing T cell lines do not secrete IL-2, IFN-γ, and lymphotoxin[46]. They have further shown that activation of antigen-specific B cells by T helper cells appears to require IL-4, and those that do not produce IL-4 fail to induce antigen-specific B cell responses.

LYMPHOKINES

OVERVIEW

Lymphokines are non-antigen-specific, hormone-like polypeptides produced by cells of the immune system[47]. They include interleukins, interferons, hematopoietic colony-stimulating factors, macrophage-modulating factors, and chemotactic factors[48]. Although they perform many diverse functions, most can be grouped into one of four categories: growth factors, activation and/or differentiation factors, lymphotoxins, and chemotactic factors[47]. These categories are by no means mutually exclusive in that it is

possible for a lymphokine to have more than one function; for example, IL-4 both activates resting B cells[49] and promotes the growth of certain T helper cell lines[50]. Like the hormones of the endocrine system, lymphokines are active in concentrations ranging from 10^{-10} to 10^{-12} M[48] and have receptors that are highly specific; for example, the high affinity IL-2 receptor has a dissociation constant of 5-20 x 10^{-12} M[51]. Given all of this, it is not surprising that the study of lymphokines is one the leading fields of research in cellular and molecular immunology.

INTERLEUKIN 1 (IL-1)

In the late 1940s it was first suggested that fever was initiated by a soluble factor produced and released by activated macrophages[52]. A protein with similar pyrogenic properties appropriately named endogenous pyrogen (EP) was demonstrated in the plasma of rabbits made febrile by injection of bacteria[53]. In the early 1970s Gery et al. reported that macrophages released one or more mitogenic substances which they called lymphocyte activating factor (LAF) that greatly potentiated the response of T lymphocytes to mitogens such as PHA but did not stimulate B cells in the absence of T cells[54,55,56]. Initial characterization of both EP and LAF revealed that agents which stimulated production of EP also stimulated LAF release, EP and LAF had similar kinetics for release, and that the two had nearly identical molecular weights and isoelectric points[53]. In the late 1970s and early 1980s Rosenwasser et al. reported that highly purified leukocytic pyrogen (also known as EP) replaced the soluble products of murine peritoneal exudate cells in the T cell proliferative response to antigen[57] and enhanced the murine thymocyte proliferation response to PHA[58] and thus established that EP and LAF were indeed the same molecule.

Interleukin 1 was first purified by Blyden and Handschumacher who found that human LAF had an apparent molecular weight of approximately 13,000 daltons[59]. Subsequent studies based on IL-1 obtained from human, mouse, and mouse macrophage cell lines have revealed a molecular weight of between 12,000 and 16,000 daltons[60]. Mizel and Mizel using culture fluid from the macrophage cell line P388D.1 first purified murine IL-1 to apparent homogeneity[60]. They noted a molecular weight of 14,000 daltons, three charged species which they termed α, β, γ, and a biological activity range within 10^{-11} to 10^{-10} M. The complementary DNAs (cDNA) for both murine and human IL-1 have recently been cloned, expressed, and sequenced[61,62]. The murine IL-1 cDNA codes for a polypeptide precursor of 270 amino acids[61] with the biologically active polypeptides at least 127 amino acids long and derived from the carboxyl terminus of the 270 amino acid precursor[63]. Two distinct but distantly related cDNAs (IL-1α and IL-1β) encoding for human IL-1 have been described[62]. Dower et al. have shown the IL-1 binds to a plasma membrane receptor with an apparent affinity of approximately 0.2-2 $\times 10^{-10}$ M and molecular weight of about 80,000 daltons[64,65]. Bird and Saklatvala[66] , Dower et al. [67], and Kilian et al.[68] have all independently shown that the IL-1 receptor on fibroblasts and on T cells binds both IL-1α and IL-1β.

In addition to its role as a mediator of the acute-phase response, IL-1 acts on a number of cells in the immune system including B cells and T cells[69]. Lipsky et al. using antiserum against human leukocytic pyrogen showed that IL-1 plays a necessary role in B cell proliferation and in the generation of immunoglobulin-secreting cells[70]. Howard et al. noted that IL-1 and IL-4 acted synergistically in the proliferation of anti-IgM activated B cells[71]. Gillis and Mizel observed that for certain T cell tumor lines IL-1 was necessary for the production of IL-2[26]. Kaye et al. using the cloned helper T cell line D10 found

that IL-1 induced IL-2 receptor expression[27]. These findings implicate IL-1 as one of the key mediators of the immune response.

INTERLEUKIN 2 (IL-2)

Interleukin 2, originally termed TCGF,was first produced by stimulating normal human peripheral blood lymphocytes with PHA[72]. Shortly after the initial description of TCGF, several investigators reported the long term growth of normal human T cells[73] and tumor-specific cytotoxic T cells[74] using media supplemented with this factor. Using the tumor-specific cytotoxic T cell line (CTLL) Gillis et al. were able to develop a sensitive microassay for TCGF[75]. Biochemical characterization of IL-2 from mitogen-stimulated human, murine, and rat lymphocytes soon followed. Interleukin 2 from human and rat lymphocytes was found to have a molecular weight (on gel filtration) of approximately 15,000 daltons[76] while murine IL-2 had a molecular weight of 30,000 daltons[77]. Taniguchi et al. using a cDNA coding for human IL-2 showed that after processing mature human IL-2 contains 133 amino acids and a calculated molecular weight of 15,420.5 daltons[78]. The observation that murine IL-2 has an apparent molecular weight twice that of human and rat has been attributed to noncovalent dimerization of a 15,000 dalton subunit[79].

The findings that the mitogenic effect of IL-2 was strictly concentration dependent[75] and that T cells activated by either lectins or antigens absorbed IL-2 in a cell concentration dependent fashion[80] suggested that the binding and removal of IL-2 might be receptor mediated[81]. Robb et al. using internally radiolabeled IL-2 in a series of competitive binding experiments were the first to show that IL-2 interacted with activated T cells through a high affinity receptor[51]. With the monoclonal antibody anti-Tac Leonard et al. were able to partially purify and characterize the human IL-2 receptor[82]. They found that the receptor was a glycoprotein with a molecular weight of 47,000-53,000 daltons. Reports

of monoclonal antibodies directed against murine (7D4, AMT-13)[83,84] and rat[85] IL-2 receptors soon followed. Both monoclonal antibodies against the murine IL-2 receptor precipitated a protein with an apparent molecular weight of 50,000-60,000 daltons; however, only AMT-13 was able to inhibit IL-2 binding. These results suggest that AMT-13 binds to the active site on the murine IL-2 receptor while 7D4 bind to a distal epitope or possibly another protein that is physically associated with the IL-2 receptor. Cloning of a cDNA encoding the human IL-2 receptor was independently undertaken by Leonard et al.[86] and Nikaido et al.[87]. Both groups found two mRNAs hybridzing to the cDNA clone and a fairly identical sequence. At about the same time Robb et al. reported the presence of high and low affinity IL-2 receptors[88]. This finding helped explain the discrepancy that arose when quantitative binding studies using radiolabeled IL-2 and anti-Tac indicated that there were far more Tac binding sites than IL-2 binding sites. Robb et al. found that the low-affinity receptors had an association constant greater than 5,000 times lower than the initially described high-affinity receptor. Robb, in a definitive study which showed the conversion of low-affinity receptors to high-affinty receptors following fusion of cell membranes, that the same Tac protein can act either as a high or low affinity receptor[89]. Kondo et al. in an elegant synthesis of the literature on the IL-2 receptor have proposed that a binary complex between the IL-2 receptor and another protein would constitute a high-affinity receptor[90]. In their model, a high-affinity receptor is the ternary complex of IL-2, the IL-2 receptor, and a "converter" protein.

Like interleukin 1 IL-2 has a number of roles in the immune response. Although primarily associated with T cell proliferation, IL-2 also directly or indirectly plays a role in the proliferation and maturation of other cell types[79]. Henney et al. found that IL-2 augmented natural killer activity[91]. Mond et al.

have shown that affinity purified IL-2 caused large B cells to proliferate[92]. Howard et al. noted that IL-2 induced certain antigen reactive T cell lines to secrete low levels of IL-4[93]. Kasahara et al. using IL-2 were able to induce peripheral T cells to secrete IFN-γ[94]. And Kelso et al., in a recent report on the regulation of GM-CSF production, observed that IL-2 preferentially induced certain T cell clones to secrete only GM-CSF as compared to GM-CSF and IL-3 when stimulated with lectins such as Con A.[95]

INTERLEUKIN 3 (IL-3)

Interleukin 3 is one of two, the other being GM-CSF, colony stimulating factors which are active on early (pluri- or multipotential) precursors of hemopoietic stem cells[96]. It was initially identified as a lymphokine capable of inducing the enzyme 20α-hydroxysteroid dehydrogenase (a specific marker of mature T cells) in cultures of the splenic lymphocytes from athymic mice[97]. In addition to being secreted constitutively by the myelomonocytic leukemia cell line WEHI-3, T cells and T cell tumors produce IL-3 upon stimulation[98]. Using WEHI-3-conditioned media Ihle et al. purified IL-3 to apparent homogeneity[99]. They noted a molecular weight of 28,000 daltons by gel electrophoresis and an activity of 1.79 x 10^{-12} M. Fung et al. using mRNA from WEHI-3 cells were able to clone and sequence the cDNA coding for murine IL-3[100]. Sequence analysis of the cloned cDNA revealed that it coded for a precursor polypeptide of 166 amino acids. Yokata et al. cloning the cDNA for mast-cell growth-factor found a sequence identical to that of IL-3[101].

A broad range of biological activities have been attributed to IL-3 including multi-colony stimulating factor, hematopoietic growth factor, burst-promoting activity, FDC-P cell line stimulating factor, mast cell growth factor, histamine-producing cell stimulating factor, and Thy 1-inducing activity[100].

INTERLEUKIN 4 (IL-4)

The existence of a T cell derived B cell growth factor was first implied from a study by Howard and her co-workers[102]. In that study normal mouse B cells were propagated with medium supplemented with a T cell-hybridoma-derived supernatant. Howard et al., using a phorbol myristate acetate (PMA) induced EL-4 supernatant, first identified a B cell growth factor (BCGF) as distinct from IL-2[103]. Ohara et al. using trimethylsilyl-controlled pore glass beads (TMS-CpG) and reverse-phase high-performance liquid chromatography (RP-HPLC) were able to partial purify IL-4 and noted an apparent molecular weight of 18,000 and 21,700 daltons when analyzed by isoelectric focusing and gel filtration-HPLC, respectively[104]. Using IL-4 purified in this manner Ohara and Paul were able to establish a rat-mouse hybridomas that secreted anti-IL-4 antibodies (11B11 and 18F10)[105]. Grabstein et al. purified IL-4 to homogeneity and found a molecular weight of 18,400 daltons by gel electrophoresis[106]. The cDNA clone that encoded for murine IL-4 was isolated, expressed, and characterized independently by Noma et al.[107] and Lee et al.[108]. Both groups noted that the murine IL-4 gene coded for a precursor polypeptide of 140 amino acids. Yokota et al. recently characterized the cDNA clone encoding for human IL-4[109]. They found that the human IL-4 gene encoded for a 153 amino acid polypeptide precursor and that on the genomic level human and murine IL-4 shared extensive homology. Although there are presently no definitive reports concerning the IL-4 receptor, Mishra et al. have reported that a monoclonal antibody directed against the lymphocyte function-associated antigen 1 (LFA-1) α-chain, a cellular adhesion molecule, mimics some of the biological effects of IL-4 suggesting that LFA-1 may be the receptor for IL-4 or a receptor-associated molecule involved in IL-4 cell signaling[110].

Interleukin 4 possess a wide variety of biological activities. In addition to acting synergistically with IL-1 in enhancing proliferation of anti-IgM activated B cells, Noelle et al. have observed that IL-4 increased expression of class II MHC products on resting B cells[111]. Rabin et al. noted that a preculture with IL-4 sped the entry of those cells into S phase when they were subsequently cultured with IL-4 and anti-IgM. They also observed that pretreatment of resting B cells with IL-4 resulted in a substantial increase in cell volume[49]. Vitetta et al. found that IL-4 in addition to being a B cell growth factor is also a B cell differentiation factor[112]. They noted that IL-4 induced the secretion of IgG1 and inhibited the secretion of IgG3 in lipopolysaccharide (LPS)-stimulated B cells. Coffman et al. noted a similar finding concerning IgE production[113,114]. A recent report by Finkelman et al. concerning the suppression of an *in vivo* polyclonal IgE response by the anti-IL-4 antibody 11B11 further supports the observation made by Coffman et al.[115]. In addition to B cells, IL-4 has also been shown to promote the growth of T helper cell lines[50,116], and to act as a costimulant with PMA on a heterogeneous population of resting T Cells[117]. Mosmann et al. have also observed that IL-4 costimulates with IL-3 to increase the proliferation of certain mast cell lines[118].

GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF)

Like IL-3, GM-CSF is active on early precursors of hemopoietic stem cells. Lopez et al. have recently reported that in addition to stimulating neutrophil progenitor cells GM-CSF also activates differentiated neutrophils[119]. They noted enhanced antibody-dependent, cell mediated cytotoxicity when mature neutrophils were cultured with GM-CSF. Kelso et al. noted another difference between IL-3 and GM-CSF; whereas, IL-3 is solely secreted by activated T cells, GM-CSF is also produced by macrophages, fibroblasts, and endothelial cells[95]. Gough et al. using a partial NH_2-terminal amino acid

sequence from purified mouse lung-conditioned medium were able to produce a cDNA clone which they used to isolate a unique gene encoding for GM-CSF[120]. They noted that the gene coded for a messenger RNA of 1,200 nucleotides, a polypeptide of 118 amino acids, and had no structural similarity to IL-3. In addition to the aforementioned activities, Handman and Burgess reported that when peritoneal macrophages were treated with GM-CSF they were able to kill intracellular *Leishmania tropica* that were hitherto multiplying within the macrophage[121]. Dessein et al. noted eosinophil cytotoxicity was marked enhanced in the presence of GM-CSF[122]. Grabstein et al. noted that GM-CSF potentiated induction of antibody secretion by activating an accessory function of macrophages[123] and induced macrophage tumoricidal activity[124]. Finally, Woods et al. have found that GM-CSF can induce proliferation in a TCGF dependent T cell line[125].

INTERFERON-GAMMA (IFN-γ)

Interferons are secreted proteins which have immunomodulatory and antitumor properties[126]. There are three major classes of interferons: IFN-α (leukocyte or fast) , IFN–β (fibroblast or slow), and IFN–γ (immune)[127]. Unlike IFN-α or IFN-β, IFN-γ is acid labile, does not cross-react with antisera prepared against IFN-α or IFN-β, and is generally produced in cultures by mitogenically stimulating lymphocytes[126]. Using a cDNA clone prepared from gel-fractionated IFN-γ mRNA, Gray et al. were able to isolate, characterize, and express the cDNA sequence encoding for the IFN-γ gene[126]. They found that the DNA coded for a 166 amino acid polypeptide including a 20 amino acids signal peptide. Taking this into account Gray et al. calculated that the processed protein not accounting for post-translatioal modifications had a molecular weight of 17,110 daltons.

Interferon-gamma has a broad range of biological activities. Blalock et al. noted that IFN-γ has both anticellular and antiviral activity[128]. Catalona et al. found that IFN-γ is a potent stimulator of antibody-dependent cell-mediated cytotoxicity (ADCC) and of natural killer cytotoxicity[129]; while, Wallach noted that IFN-γ induced resistance to the killing by NK cells[130]. A number of investigators have all independently shown that IFN-γ induces expression of class II MHC products on macrophages[131,132,133] and on endothelial cells[134]. Another immunomodulatory function has recently been ascribed to interferon-γ. Coffman and Carty[113] and Rabin et al.[135] have observed that the IL-4 polyclonal IgE response and the IL-4 response on resting B cells can be inhibited by IFN-γ. Mond et al. have recently shown that IFN-γ suppresses the IL-4 induced augmentation of class II MHC product expression[136]. Finally, Grabstein et al. noted that IFN-γ also induced peripheral blood monocytes to exhibit tumoricidal activity against a malignant melanoma cell line[124].

LYMPHOTOXIN (LT)

Lymphotoxin is a lymphocyte derived glycoprotein possessing direct cytolytic and cytostatic activities for a variety of cells was first described by Ruddle and Waksman[137]. They noted that lymph node cells from inbred rats with delayed hypersensitivity to tuberculoprotein, bovine gammaglobulin, and egg albumin produced progressive destruction of monolayers of rat embryo fibroblasts in tissue culture[138]. Although some investigators have reported a number of different molecular weight lymphotoxins[139], Gray et al. in cloning and expressing the cDNA encoding human lymphotoxin reported that lymphotoxin was encoded by a single gene[140]. They found that the lymphotoxin gene coded for a precursor polypeptide of 205 amino acids which after processing but before glycosylation was thought to have 171 amino acid and a molecular weight of 18,600 daltons.

A number of different biological activities has been ascribed to lymphotoxin. It has been shown to cause inhibition of tumor cell proliferation[141]. Sawada and Osawa implicated lymphotoxin as the effector molecule in T cell mediated cellular cytotoxicity[142]; while, Wright and Bonavida found that a lymphotoxin-like molecule mediated lysis of natural killer cell targets[143]. However, Green et al. recently readdressed this issue and found that human cytotoxic T cells produced cytotoxins that were antigenically distinct from lymphotoxin[144]. Kondo et al. observed that antiserum against lymphotoxin inhibited ADCC and hence suggested that lymphotoxin was the mediator of ADCC[145]. Stone-Wolff et al.[146] and Lee et al.[147] have both recently shown marked synergistic enhancement of cytotoxicity when lymphotoxin is added to interferon-γ.

EXPERIMENTAL HYPOTHESIS

The growth and proliferation of mammalian cells are controlled in part by polypeptide hormones found in the serum[148]. Malignant or transformed cells appear to require no or lower concentrations of growth-controlling factor(s) as compared with normal or untransformed cells[148]. It has therefore been suggested that cells could become malignant via the aberrant production of polypeptide growth-controlling factor(s) acting in an autocrine manner through functional cell membrane receptors resulting in uncontrolled proliferation[149]. Recently, the "autocrine hypothesis" has been further defined in that malignant transformation may be the result not only of excessive production, expression, and action of positive autocrine growth factors but also the failure of cells to synthesize, express or respond to specific negative growth factors that they normally release to control their own growth[150].

Interleukin 2, originally termed T cell growth factor, has been shown to promote the proliferation of any IL-2 receptor positive T cell[79]. It has been shown that the T helper cell line D10 apparently secretes large amounts of IL-2 (as determined by its ability to stimulate an IL-2 dependent cell line and by the observation that its autocrine proliferation is inhibited by a monoclonal anti-IL-2 antibody) upon crosslinking of its antigen-class II MHC products receptor yet fails to proliferate[27,34]. Furthermore, D10 cells have to been shown to both respond to IL-2 derived from the T cell hybridoma AOFS[34] and to express functional IL-2 receptors[27]. Thus in the presence of both functional IL-2 and IL-2 receptors this clone fails to proliferate.

The goal of the experiments described in this thesis is to determine the mechanisms involved in this paradox. The growth factor(s) found in the stimulated supernatants from D10 cells and AOFS cells will be further characterized in order to ascertain why these two supernatants have different

biological activities yet both apparently contain IL-2. It is possible that the supernatant from the transformed cell line contains a growth-factor(s) that is(are) absent from the normal cell lines' supernatant. Likewise, it is possible that the D10 supernatant has a negative growth-factor(s) absent from the AOFS supernatant that prevents its uncontrolled proliferation.

biological activities yet both apparently contain IL-2. It is possible that the

supernatant from the transformed cell line contains a cytokine (factor) that is

absent from the normal cell-line supernatant ... and when it is added

that the D10 supernatant has a proliferative effect ... D10 cells in ...

supernatant that showing of ...

MATERIALS AND METHODS

Animals. The mice used in these experiments, BALB/cByJ and AKR/J, were obtained from The Jackson Laboratory.

Cloned T-Cell Lines. The AKR/J cloned T-cell line D10.G4.1 (D10) has been described extensively[23,24,27,34]. It is specific for the hen white protein conalbumin (Sigma Chemical Co. St. Louis, MO) in the context of I-A^k and also responds to the monoclonal anti-T cell receptor antibody 3D3 (see below). The BALB/cByJ cloned T cell lines 5.5 and 5.9.24 have been described in detail elsewhere[151,152,153]. They are specific for hen ovalbumin (Sigma Chemical Co. St. Louis, MO) and I-E^d or I-A^d, respectively. These cloned T cell lines were maintained at 37°C in a 5% CO_2 atmosphere by feeding with syngeneic mitomycin C-treated spleen cells and specific antigen (100 µg/ml) every one to three weeks in Click's EHAA medium[154] (Irvine Scientific, Santa Ana, CA) supplemented with 10% fetal bovine serum (Irvine Scientific, Santa Ana, CA) and 5% TCGF-containing supernatant of concanavalin A (Con A) (Pharmacia Inc., Piscataway, NJ)-treated rat spleen cells[155]. Between feedings with spleen cells and antigen, the T cell lines were fed at intervals with the same medium.

Antibodies. *Monoclonal murine IgG1 anti-D10-receptor antibody (3D3).* The monoclonal antibody was produced and assayed as described[34]. It was used as a saturated ammonium sulfate (SAS) precipitated, Protein A purified diluted ascitic fluid.

Monoclonal rat IgG1 anti-murine-BSF-1/IL-4 antibody (11B11). Hybridoma cells were a kind gift from J. Ohara and W. E. Paul (National Institutes of Health, Bethesada, MD) and this antibody has been extensively described elsewhere[105]. Antibody was used as a SAS precipitated ascitic fluid at a final concentration of 1: 200.

Monoclonal rat IgG2a anti-murine-IL-2 antibody (S4B6). Hybridoma cells were a kind gift from T. Mosmann (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA) and this antibody has been extensively elsewhere[118]. Antibody was used as serum-free culture supernatant at a final concentration of 1: 4 unless otherwise noted.

Culture Supernatants. *Interleukin 1* Murine IL-1 was prepared as described[27] by stimulating the murine macrophage cell line P388.D1 with 1μg/ml of lipopolysaccharide (Difco Labs, Detroit, MI) for twenty-four hours.

Recombinant-derived murine IL-1α. Murine IL-1α was a kind gift of S. Gillis (Immunex Corp. Seattle, WA).

Recombinant-derived human IL-2. Human IL-2 was purchased from AMGen Biologicals (Thousand Oaks, CA).

Recombinant-derived murine IL-2 Murine IL-2 was a kind gift of S. Gillis (Immunex Corp. Seattle, WA).

Affinity purified BSF-1/IL-4. A high titered, BSF-1/IL-4 containing supernatant was kindly provided by L. Greenbaum (Yale University School of Medicine, New Haven, CT). Briefly, D10 cells were incubated for twenty-four hours at a concentration of one million cells per milliliter in serum-free EHAA medium containing 1% HB101 (Hara Medium, Berkeley, CA) and 2.5μg/ml of Con A. Supernatants were collected and passed over an anti-BSF-1/IL-4 affinity column. The bound material was eluted with 0.1% trifluoracetic acid. Its biological activity was inhibited by a monoclonal anti-BSF-1/IL-4 antibody.

Recombinant-derived murine Interferon-γ. Murine IFN-γ was a kind gift of the Genentech Inc. (San Francisco, CA).

AOFS supernatants. The T cell hybridoma AOFS 21.10.9 was kindly donated by P. Marrack and J. Kappler (National Jewish Hospital, Denver, CO). Supernatants were obtained by incubating cells at an initial concentration of 5

x10[5] cell/ml with 2.5µg/ml of Con A in EHAA medium lacking serum for twenty-four hours. Con A Supernatants were then collected, filtered over a 0.22µm millipore filter (Millipore Corp., Bedford, MA).and neutralized with 20mg/ml of methyl α-D-mannoside (Sigma Chemical Co., St. Louis, MO).

D10 supernatants/Inhibitor supernatants. D10 cells at one hundred thousand (10[5]) cells per milliliter were stimulated for twenty-four hours in Click's EHAA medium containing 5% serum with either the monoclonal anti-receptor antibody 3D3 at 20ng/ml or Con A at 2.5µg/ml. Supernatant were collected and flitered over a 0.22µm millipore filter, and the Con A supernatants were neutralized with 20mg/ml of methyl α-D-mannoside. All assay using such supernatants contained 5mg/ml of methyl α-D-mannoside. Unless referred to otherwise 3D3 was used to generate the supernatants.

Bioassays. *Proliferation assays.* Cells plus supernatants were incubated together in either 100µl or 200µl volumes of Click's EHAA/5% fetal bovine serum in flat-bottom 96 well trays. After culture for twenty of forty-four hours at 37°C in a 5%CO_2 atmosphere, 1µCi (1Ci= 37GBq) of tritiated-thymidine was added to each microculture. Four hours later, cells were harvested with a PHD harvester (Cambridge Technologies, Cambridge, MA), and incorporated tritiated-thymidine of triplicate cultures were determined. Standard deviations of mean CPM incorporated were <20% and have been omitted.

IL-1 assay. IL-1 was assayed as previously described by comitogenesis of D10 cells and 3D3[27]. Such supernatants caused no growth of D10 in the absence of and were without activity in the TCGF assay.

TCGF assay. IL-2 and/or IL-4 was assayed by measuring the incorporation of tritiated-thymidine by the TCGF-dependent T helper cell line HT-2, initially described by Watson[156]. After twenty hours, microcultures of 10[4]

cells were incubated for four hours with 1μCi of tritiated-thymidine and subsequently harvested and mean CPM of triplicate cultures determined.

Inhibitor assay. Inhibitor containing supernatants were incubated for forty-eight hours with twenty thousand (2×10^4) 5.5 cells in the presence of 0.5 units of recombinant IL-2. Proliferation was measured by pulsing with 1μCi/well of tritiated-thymidine for the final four hours of the assay. Cells were subsequently harvested and mean CPM of triplicate cultured determined.

Results

The cloned murine T helper cell line D10 has previously been shown to secrete large amounts of a T cell growth factor (TCGF) when stimulated with a monoclonal antibody (3D3) specific to its T cell receptor complex[27]. D10 cells have also been shown to proliferate in response to an IL-2 rich AOFS supernatant[34] and to recombinant IL-2. However, when D10 cells are stimulated with 3D3 they fail to proliferate unless IL-1 is also added to the cultures (Table I). To further explore this observation, D10 cells were cultured with recombinant IL-2 and with a 3D3 stimulated D10 supernatant. As noted in Table 1, D10 cells only proliferated in response to recombinant IL-2. Given these paradoxical findings, the following experiments were performed in order to explain this phenomenon.

Both D10 Supernatant and AOFS Supernatant Have TCGF Activity. When D10 cells are stimulated with 3D3 in a twenty-four hour culture they secrete a factor(s) which has TCGF activity as measured by bioassay of the supernatant on the TCGF dependent cell line HT-2 (Fig. 1). Likewise, when the T cell hybridoma AOFS is stimulated with the mitogenic lectin Con A a twenty-four hour supernatant also stimulates the proliferation of HT-2 cells. Thus, D10 supernatant and AOFS supernatant contain a factor(s) possessing TCGF activity.

AOFS Supernatant Contains IL-2 and IL-4 While D10 Supernatant Contains Only IL-4. The preceding experiment revealed that both supernatants had TCGF activity. Given that HT-2 cells respond to both IL-2[106], IL-4[50], and GM-CSF[125] it was necessary to determine which factor(s) in each supernatant was causing the growth of the HT-2 cells. Using monoclonal antibodies with specificities against either IL-2 (Table II) or IL-4 each supernatant was tested. (Presently, there are no monoclonal antibodies specific for GM-CSF).

Table I. Both D10 and HT-2 Cells Proliferate in Response to Recombinant IL-2 While Only HT-2 Cells Proliferate in Response to D10 Supernatant.

Stimulus	D10[1]	HT-2[2]
-	1030	563
+3D3[3]	3096	-
+IL-1[4]	1742	-
+3D3+IL-1	53058	-
+RIL-2[5]	36987	178610
+3D3-stimulated D10 S/N[6]	3903	97402

[1]D10 cells at 20,000 cells/well were incubated in triplicate culture in the presence of the above stimuli for forty-eight hours. Proliferation was measured by pulsing with tritiated thymidine for the last four hours of the assay. Mean ±SD (omitted from table) were calculated for each triplicate culture.

[2]HT-2 cells at 10,000 cells/well were incubated in triplicate culture in the presence of the above stimuli for twenty-four hours. Proliferation was measured by pulsing with tritiated thymidine for the last four hours of the assay. Mean ±SD (omitted from table) were calculated for each triplicate culture.

[3]20ng/ml of 3D3.

[4]35 P388D1 IL-1 rich supernatant.

[5]5.0 units of recombinant IL-2.

[6]D10 cells at 10^5 cells/ml were stimulated with 20ng/ml of 3D3 for twenty-four hours. Supernatant (S/N) was collected, filtered, and used at a concentration of 50%.
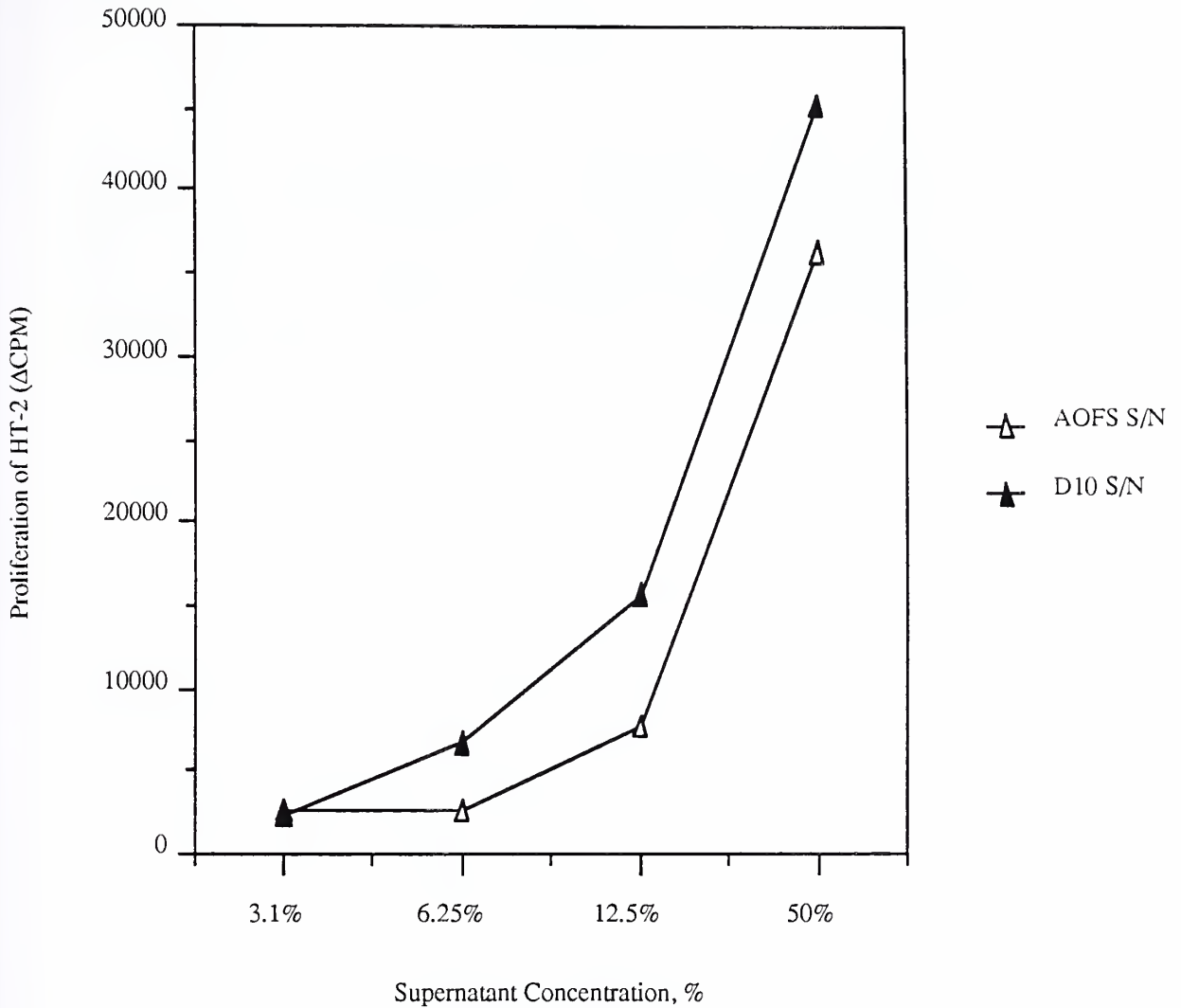
Figure: 1

Figure 1: HT-2 cells at 10,000 cells/well were incubated in triplicate culture for twenty-four hours with varying concentrations of TCGF containing supernatants. Proliferation was measured by pulsing for the last four hours of the assay with 1μCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.

Table II. The Monoclonal Antibody S4B6 Inhibits the Proliferative Response of HT-2 Cells to Recombinant Murine Interleukin 2.

Concentration of RIL-2	Control	+S4B6
1.25 Units	50060	37174
0.08 Units	2268	137

HT-2 cells at 10,000 cells per well were incubated in triplicate culture for twenty-four with murine recombinant IL-2 in the presence or absence of a 1:2 dilution of S4B6 culture supernatant. Proliferation was measured by pulsing with 1μCi/well of tritiated thymidine for the last four hours of the assay. Mean ± SD (<20%, omitted from table) was calculated for each triplicate culture. Response reported in ΔCPM.

Treatment of the AOFS supernatant with either the monoclonal antibody to IL-2 or to IL-4 resulted in decreased proliferation by the HT-2 cells (Fig. 2). When treated with both antibodies together the TCGF containing activity in the AOFS supernatant was essentially removed. When the D10 supernatant was cultured with the same panel of monoclonal antibodies, only treatment with the monoclonal antibody specific to murine IL-4 resulted in a decreased proliferative response by the HT-2 cells (Fig. 3). Quantification of the TCGF activity in the AOFS supernatant revealed that it contained 25 units/ml of IL-2 and approximately 8 units/ml of IL-4; while, the TCGF activity of D10 supernatant was due to the presence of about 45 units/ml of IL-4. In summary, AOFS supernatant contains both IL-2 and IL-4 while D10 supernatant contains only IL-4.

D10 Supernatant Fails To Induce Proliferation of D10 Cells. Given that both the AOFS supernatant and the D10 supernatant caused the T helper cell line HT-2 to proliferate, the question as to whether both supernatants could cause growth in another T helper cell line was readdressed. Both an AOFS supernatant and a D10 supernatant were titrated on D10 cells. The AOFS supernatant caused proliferation on D10 that approached background when diluted (Fig. 4). On the other hand, D10 supernatant which caused analogous proliferation to that of the AOFS supernatant of HT-2 cells induced only minimal proliferation of D10 cells (Table I and Figs.1 and 4). In summary, although D10 supernatant contains a T cell growth factor it nonetheless failed to induce a proliferative response on D10 cells.

The question that naturally arises from these observations is: what prevents the TCGF found in D10 supernatant from acting upon D10 cells? A preliminary answer emerges when one closely examines the effects on D10 supernatant on D10 cells. At high concentrations D10 supernatant does not
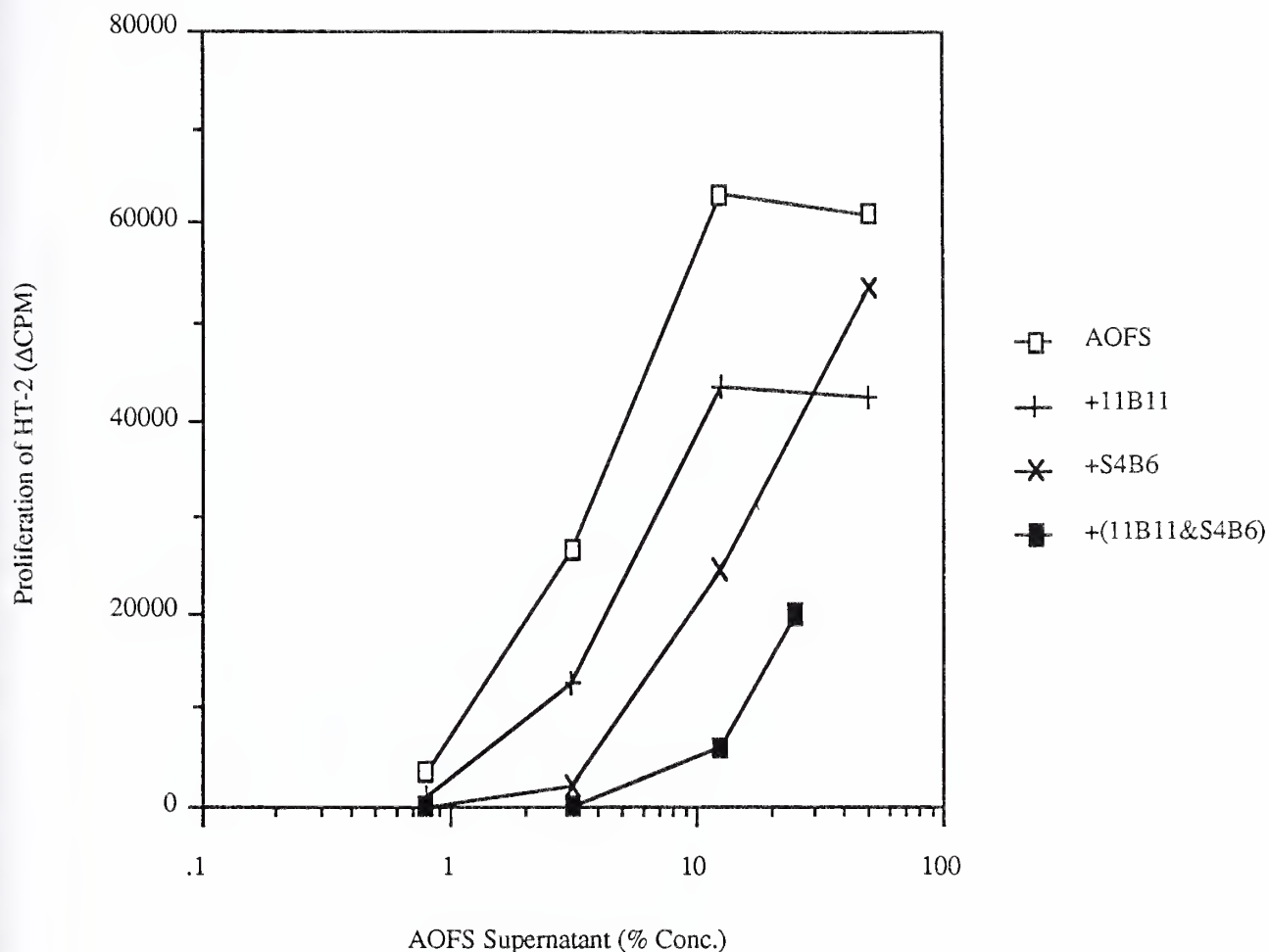
Figure: 2

Figure 2: HT-2 cells at 10,000 cells/well were incubated in triplicate culture for twenty-four hours with varying concentrations AOFS supernatant in the presence or absence of a variety of monoclonal antibodies. 11B11 is a monoclonal antibody with specificity against murine BSF-1/IL-4. S4B6 is a monoclonal antibody with specificity against murine IL-2. Proliferation was measured by pulsing for the last four hours of the assay with 1µCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.
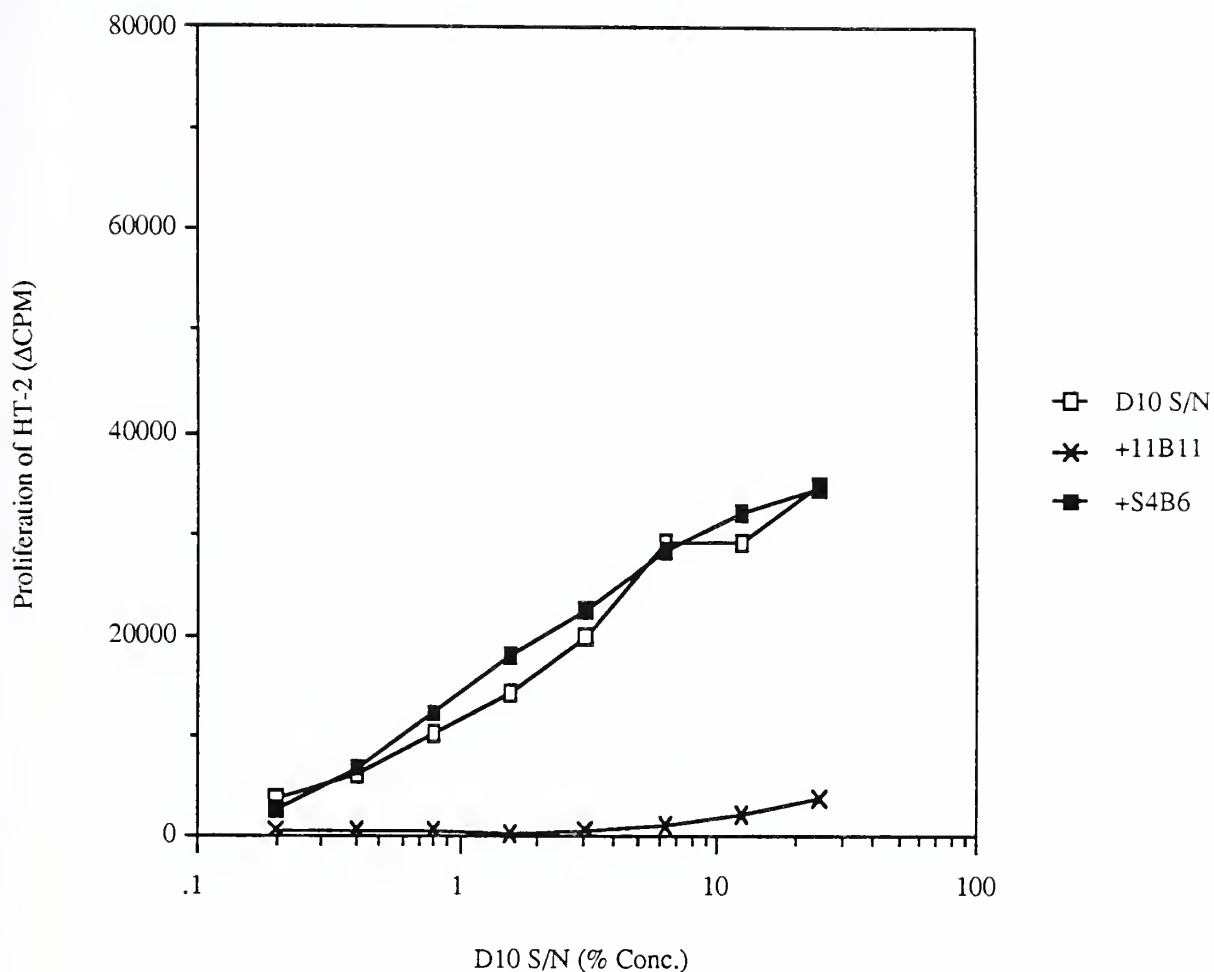
Figure:3

Figure 3: HT-2 cells at 10,000 cells/well were incubated in triplicate culture for twenty-four hours with varying concentrations D10 supernatant in the presence or absence of a variety of monoclonal antibodies. 11B11 is a monoclonal antibody with specificity against murine BSF-1/IL-4. S4B6 is a monoclonal antibody with specificity against murine IL-2. Proliferation was measured by pulsing for the last four hours of the assay with 1μCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.
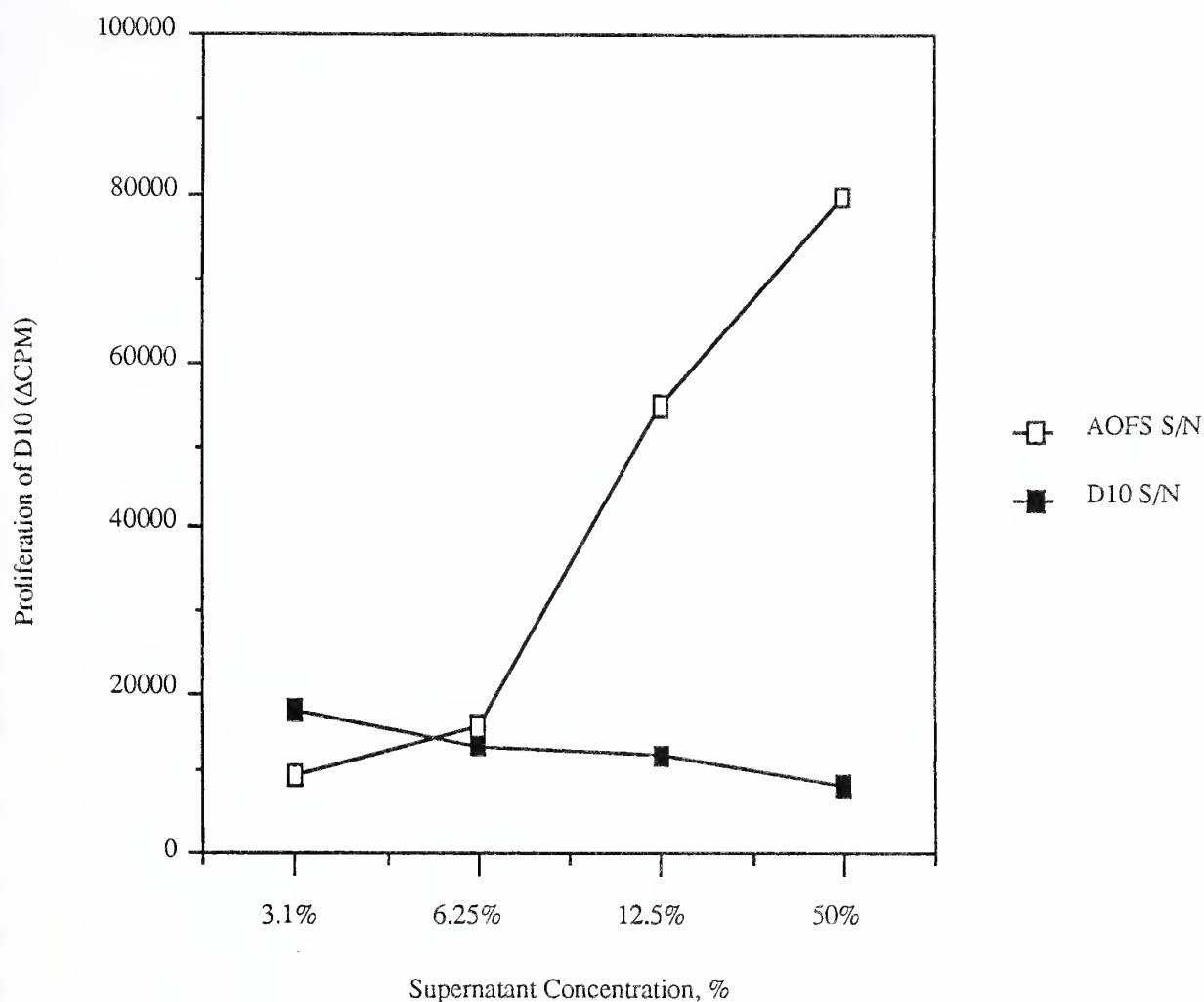
Figure: 4

Figure 4: D10 cells at 20,000 cells/well were incubated in
triplicate culture for forty-eight hours with varying
concentrations of TCCF containing supernatants.
Proliferation was measured by pulsing for the last four
hours of the assay with $1\mu Ci$/well of tritiated thymidine.
Mean ±SD (<20%, omitted from figure) was calculated
for each triplicate culture.

cause substantial growth of D10 cells; however, when the supernatant is diluted D10 cells begin to show an increasing proliferative response. These findings suggest that there is an inhibitor in D10 supernatant which is not present in AOFS supernatant that prevents autocrine stimulation.

D10 Supernatant Inhibits the Response of Other Cloned T Cell Lines to IL-2. In order to determine whether the inhibitory nature of D10 supernatant was unique to the D10 cell line other T helper cell lines were tested for their ability to respond to D10 supernatant, recombinant IL-2, and a mixture of both. The BALB/c cloned T cell line 5.5 responds well to recombinant IL-2 but not to D10 supernatant (Fig. 5). Furthermore, D10 supernatant significantly inhibits the response of these cells to recombinant IL-2. Analogous results were seen with another BALB/c T cell clone 5.9.24 (Fig. 6). In summary, D10 supernatant can significantly inhibit the proliferative response to recombinant IL-2 on a number of different T helper cell lines.

These experiment further defined some of the cellular properties of the inhibitory factor (s) in D10 supernatant. (For simplicity, the inhibitory factor (s) in D10 supernatant shall now be referred to as Inhibitor). Given that 3D3 does not react specifically with either 5.5 or 5.9.24, and that analogous results were obtained whether D10 was stimulated with either 3D3 or Con A (data not shown), the inhibitory substance is not 3D3. Furthermore, as the T helper cell clones D10 and 5.5 differ in antigen specificity and MHC restriction, and are derived from mice differing at MHC and other loci, Inhibitor is apparently not genetically restricted in its action on other cells and thus is distinct from most known suppressor factors.

Secretion of Inhibitor by D10 is Stable Over Time. To determine whether or not secretion of Inhibitor was the result of transient cell culturing conditions, the experimental protocol was repeated periodically over a time span of
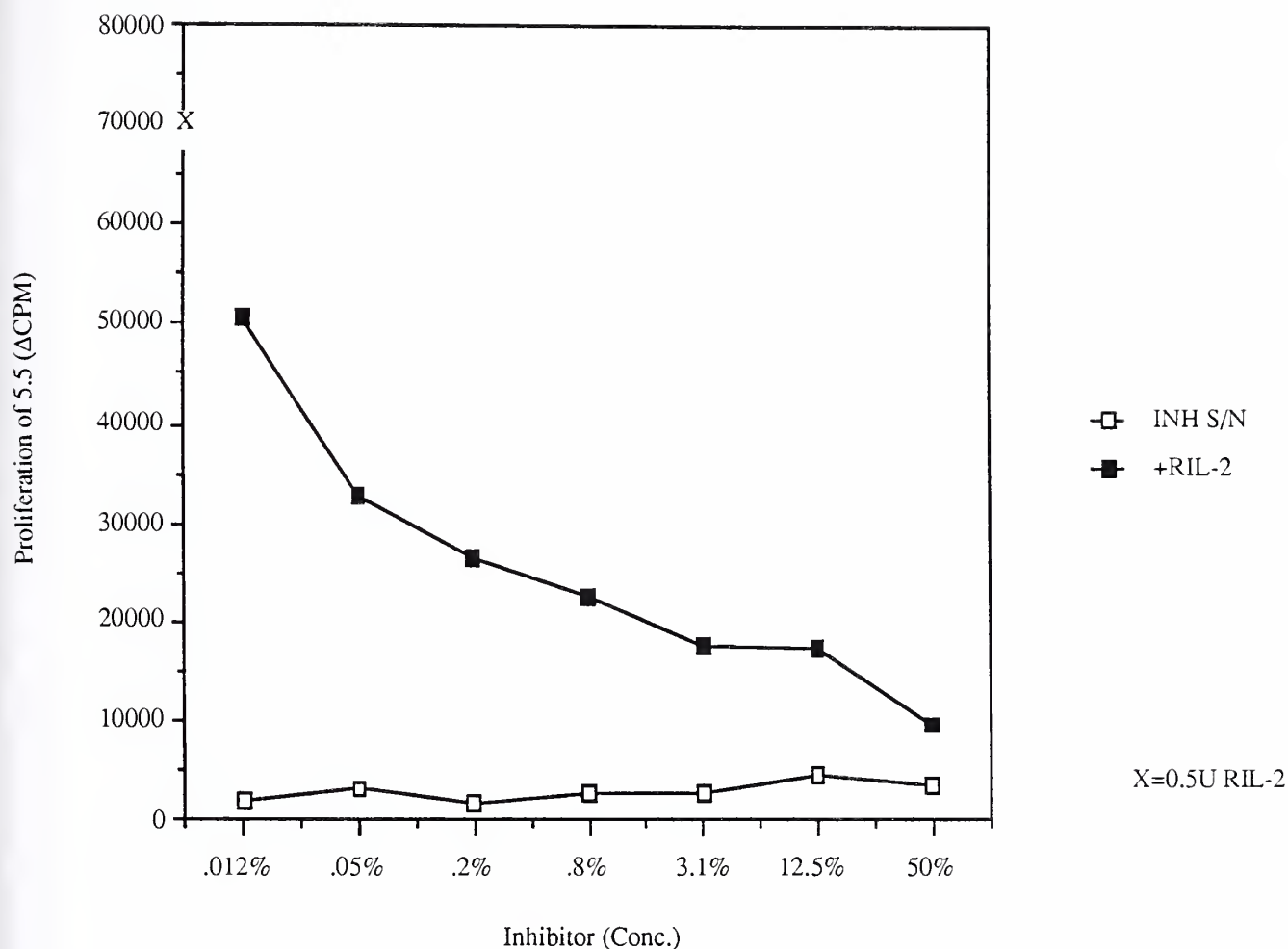
Figure: 5

Figure 5: 5.5 cells at 20,000 cells/well were incubated in
triplicate culture for forty-eight hour with varying concentrations
of Inhibitor supernatant in the presence or absence of 0.5 Units
of recombinant IL-2. Proliferation was measured by pulsing
with 1μCi of tritiated thymidine for the last four hours of the
assay. Mean ±SD (<20%, omitted from figure) was calculated
for each triplicate culture.

Figure: 6

Inhibitor Supernatant (Conc.)

Figure 6: 5.9 cells at 20,000 cells/well were incubated in
triplicate culture for forty-eight hours with varying
concentrations of Inhibitor supernatant in the presence of 0.5
Units of recombinant IL-2. Proliferation was measured by
pulsing with 1μCi/well of tritiated thymidine for the last four
hours of the assay. Mean $\pm$SD (<20%, omitted from figure)
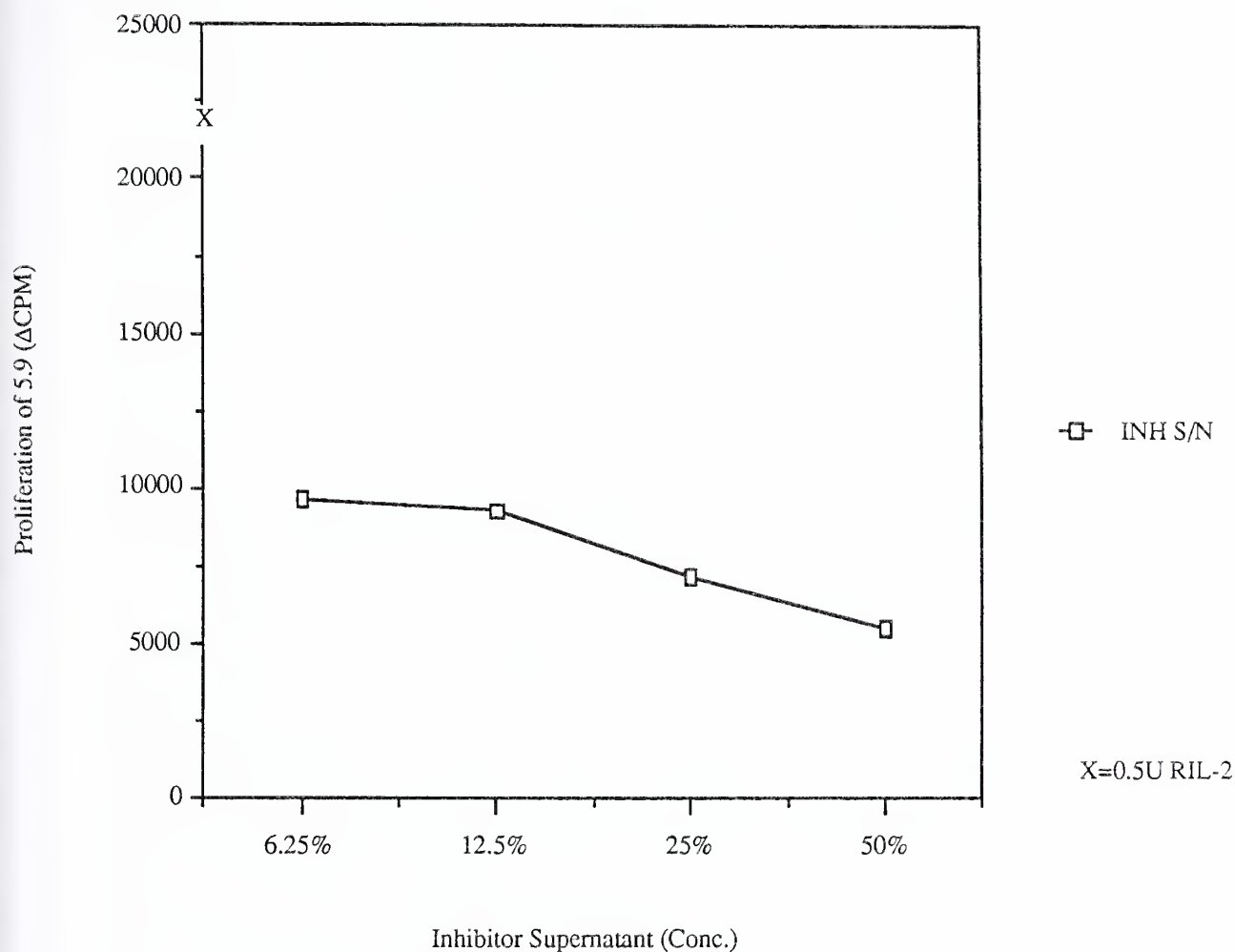was calculated for each triplicate culture.

approximately three years. Although the responsiveness of 5.5 cells to recombinant IL-2 has apparently changed Inhibitor can still significantly inhibit the proliferative response to IL-2 on the 5.5 cells (Fig. 7). Thus Inhibitor was not the result of transient cell culturing conditions.

Interferon-γ is Not Inhibitor. As previously discussed, IFN-γ has both immunoregulatory and anticellular activities. Given that both Inhibitor and IFN-γ have apparent antiproliferative properties and are T helper cell products it was necessary to determine whether Inhibitor is IFN-γ. A titration of recombinant IFN-γ in the presence of recombinant IL-2 did not result in diminution of the IL-2 proliferative response (Fig. 8). This observation combined with those of Mosmann et al.[44] and Killar et al.[46] that IL-4 producing T helper cell lines do not secrete IFN-γ provides definitive evidence that Inhibitor is not IFN-γ.

Interleukin 4 is Not Inhibitor. It may be argued that on theoretical grounds IL-4 may be the Inhibitor. Given that IL-2 and IL-4 both activate HT-2 cells it is possible (although not probable) that they use the same or similar receptors. This being correct, it would not be unreasonable to assume that these ligands would have different affinities for this presumed receptor. If so, IL-4 binding this presumed receptor may not result in activation but may occupy binding sites such that IL-2 can not signal the cell. Therefore, if the concentration of IL-4 is much higher than that of IL-2, IL-4 may act as an inhibitor of IL-2. To determine the plausibility of this assumption varying concentrations of IL-4 were cultured with IL-2 (Fig. 9). The results here show that IL-4 synergizes with IL-2 resulting in the proliferation of 5.5 cells. The finding of Robb et al.[51] that the IL-2 receptor has a very high affinity for IL-2 and the observation by Ohara and Paul[105] that anti-IL-2 receptor antibodies failed to block the biological activity of IL-4, combined with the results from this experiment strongly suggest that IL-4 is not Inhibitor.
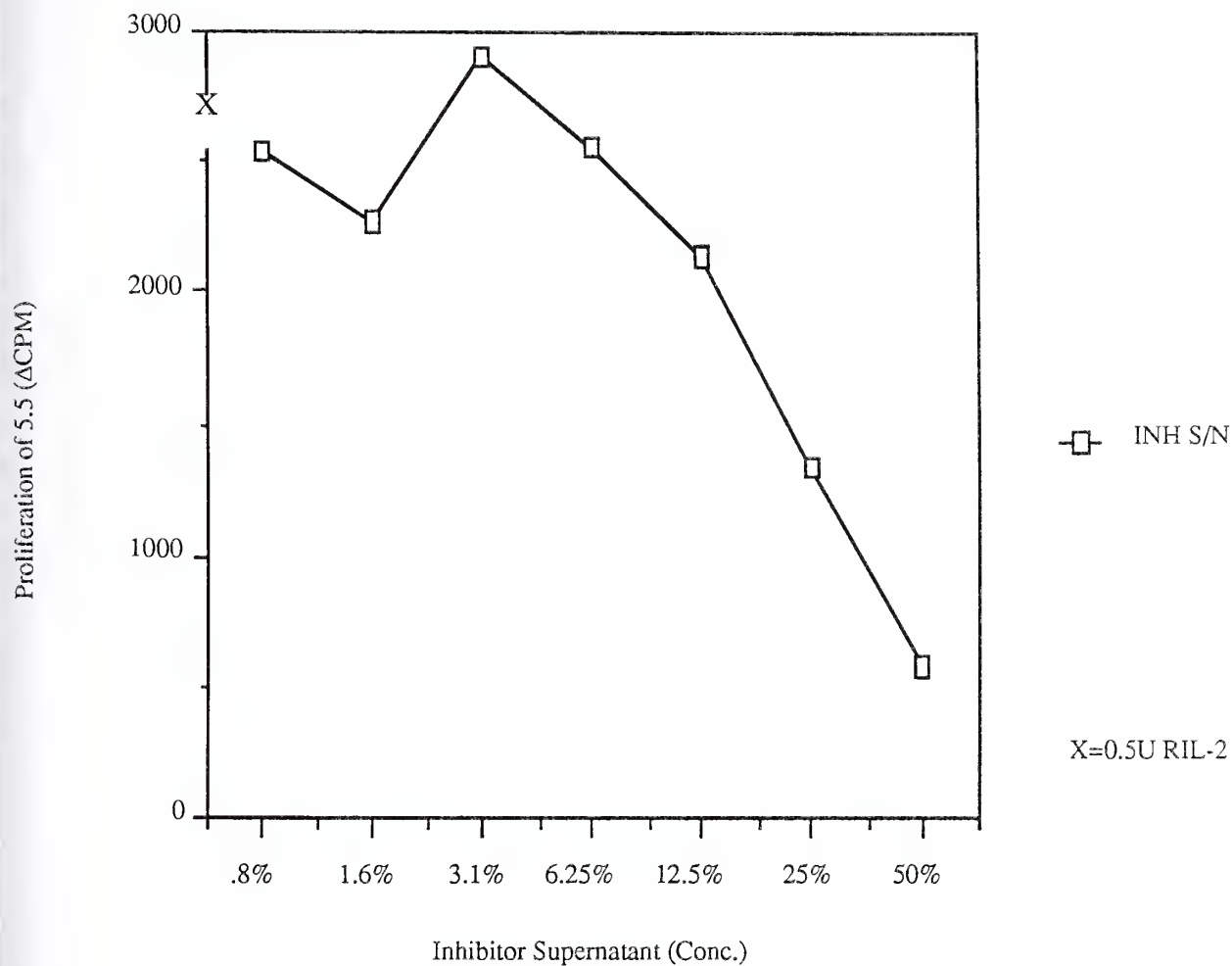
Figure: 7

Figure 7: 5.5 cells at 20,000 cells/well were incubated in
triplicate culture for forty-eight hour with varying
concentrations of Inhibitor supernatant in the presence of 0.5
Units of recombinant IL-2. Proliferation was measured by
pulsing with 1μCi of tritiated thymidine for the last four hours
of the assay. Mean ±SD (<20%, omitted from figure) was
calculated for each triplicate culture.

Figure: 8
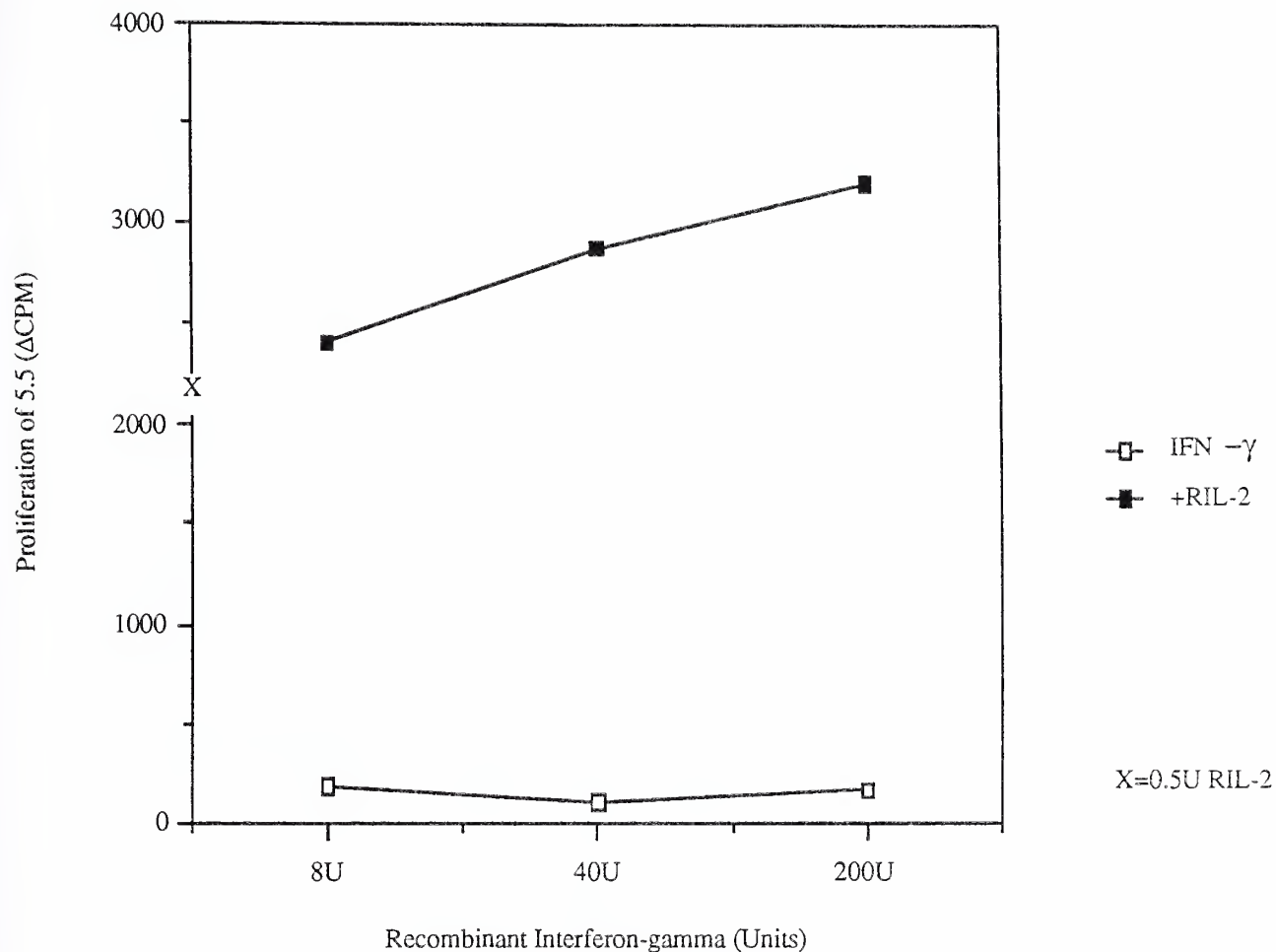
Figure 8: 5.5 cells at 20,000 cells/well were incubated in triplicate culture for forty-eight hour with varying concentrations of recombinant interferon- gamma in the presence or absence of 0.5 Units of recombinant IL-2. Proliferation was measured by pulsing with 1μCi/well of tritiated thymidine for the last four hours of the assay. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.

Figure: 9

Figure 9: 5.5 cells at 20,000 cells/well were incubated in triplicate culture for forty-eight hour with varying concentrations of affinity purified BSF-1/IL-4 in the presence or absence of 0.5 Units of recombinant IL-2. Proliferation was measured by pulsing with 1μCi/well of tritiated thymidine for the last four hours of the assay. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.

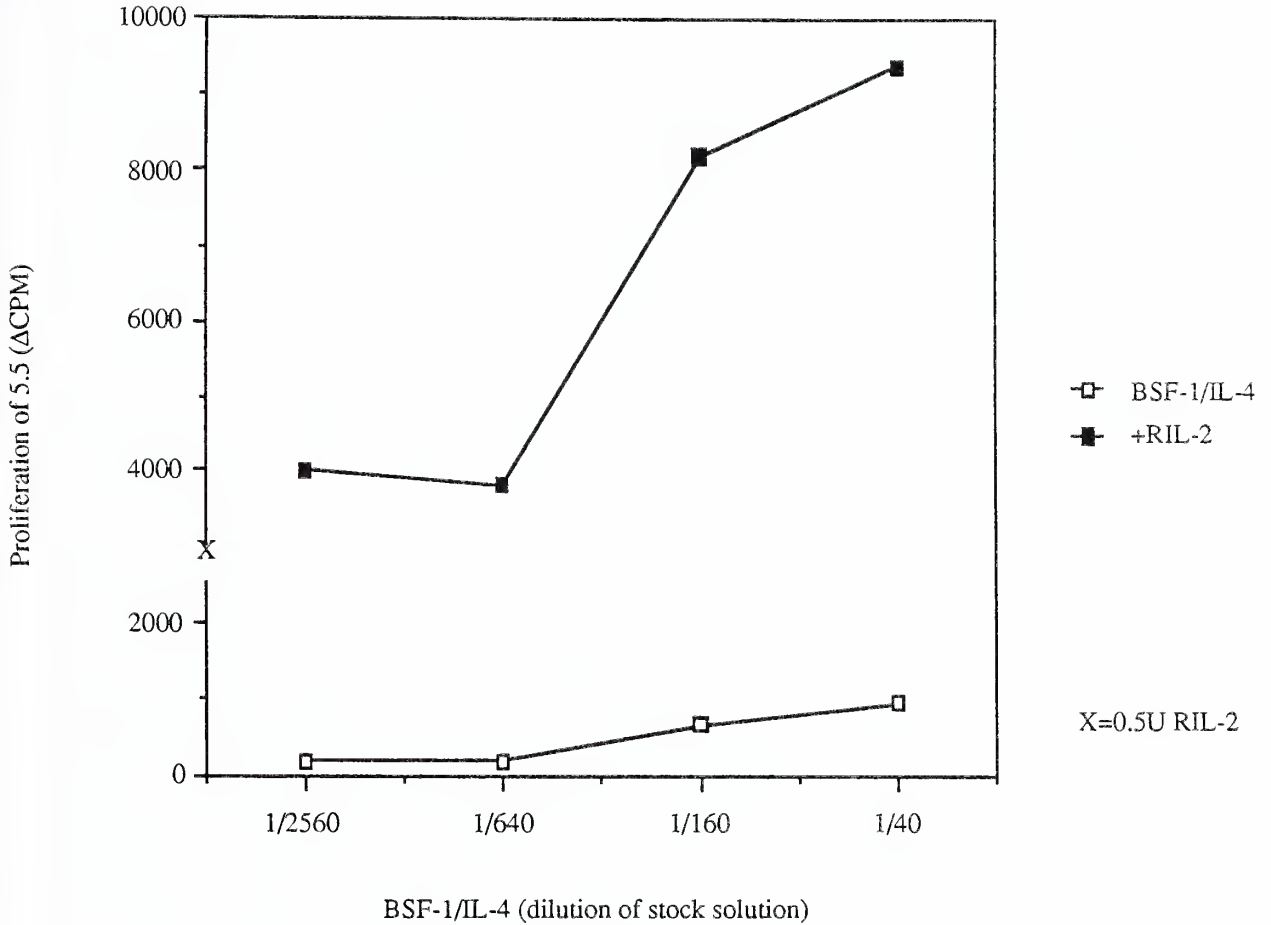**D10 Expresses Functional IL-1 Receptors.** Kaye et al.[34] noted that when D10 cells were stimulated with 3D3, the presence of either accessory cells or IL-1 was necessary to insure cellular proliferation. This observation led Kaye et al. to propose that two distinct events, namely receptor cross linking and stimulation with IL-1, must take place in order for cellular proliferation to occur. The question that logically follows from this is: by what mechanism does receptor cross linking and stimulation with IL-1 lead to proliferation of D10? As shown in Table I and Fig. 3, activation of D10 in the absence IL-1 results in secretion of IL-4 but no growth and IL-1 alone does not induce D10 to proliferate. However, if D10 cells are cultured in the presence of both D10 supernatant (as a source of IL-4) and IL-1 proliferation occurs (Fig. 10). In order to rule out the possibility that this proliferation was due to carryover 3D3, an identical experiment using a Con A induced D10 supernatant was performed. Analogous findings were noted (data not shown), thus proliferation was not caused by carryover 3D3 but the result of synergy between IL-1 and the factor(s) found in D10 supernatant. In a similar experiment done by Dr. C. Janeway, preculture of D10 cells with IL-1 followed by subsequent 3D3 stimulation also resulted in proliferation. The conclusions to be drawn from these experiments are that the factor(s) contained in D10 supernatant and IL-1 are synergistic on D10 cells, and that under its current cell culturing conditions D10 expresses functional IL-1 receptors.

Autocrine Proliferation of D10 is an IL-4 Mediated Process. The finding that D10 supernatant caused HT-2 cells to proliferate, combined with the observation that the proliferative response of D10 to 3D3 and IL-1 was greatly reduced by a monoclonal antibody with a reported specificity against murine IL-2, led to the conclusion that D10 secreted IL-2[27,34]. However, using monoclonal antibodies with more clearly defined specificities against murine IL-

Figure: 10

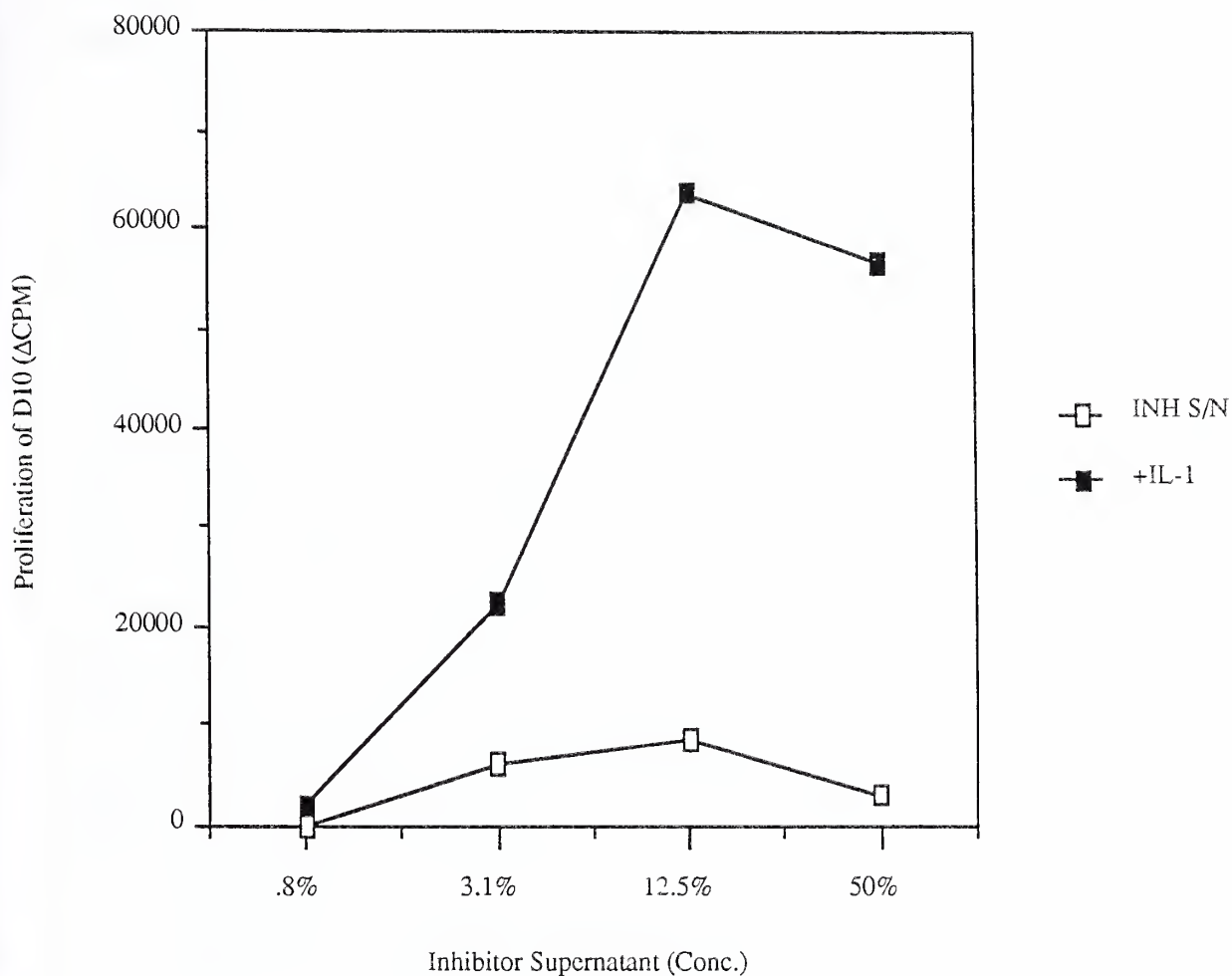Figure 10: D10 cells at 20,000 cells/well were incubated in triplicate culture for forty-eight hours with varying concentrations of Inhibitor supernatant in the presence or absence of an IL-1 rich P388D.1 supernatant. Proliferation was measured by pulsing for the last four hours of the assay with 1μCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.

2 and IL-4, it was determined that D10 supernatant contains only IL-4 (Fig. 3) and that the proliferative response of D10 to 3D3 and IL-1 was IL-4 and not IL-2 mediated (Table III). Thus, the autocrine proliferation of D10 is an IL-4 mediated process.

Interleukin 1 Augments the Response of D10 to IL-4. Given that autocrine proliferation in the D10 system is IL-4 mediated and that D10 supernatant and IL-1 are synergistic on D10 it appears that IL-1 is necessary for optimum IL-4 function. To test this hypothesis D10 cells were incubated with various concentrations of affinity purified IL-4. In the absence of recombinant murine IL-1 minimal proliferation of D10 occurred (Fig. 11). However, when affinity purified IL-4 was cultured in the presence of recombinant murine IL-1 there was substantial proliferation of D10. To test whether the proliferation of D10 was due to IL-4 and not some other factor a monoclonal antibody with specificity against murine IL-4 was used as an internal control. As shown in Fig. 11 the proliferation of D10 was substantially diminished in the presence of the antibody. An appealing explanation for this observation is that IL-1 is necessary for the expression of IL-4 receptors.

Interleukin 1 Augments Response of HT-2 to IL-4. Given that HT-2 cells respond to IL-4[50] and the observation that IL-1 augments the response to IL-4, it was thought that IL-1 also may augment the IL-4 response on the HT-2 cell line. As shown in Fig. 12 HT-2 cells behave analogously to D10. Thus, IL-1 augments the response of HT-2 cells to IL-4.

Interleukin 1 Augments the Response of D10 But Not HT-2 to IL-2. Kaye et al.[27] have previously shown that IL-1 is necessary for IL-2 receptor expression on D10. Figure 13 shows that IL-1 augments the response of D10 cells to IL-2, thus confirming the finding of Kaye et al. Figure 13 also shows that the anti-IL-4 antibody 11B11 inhibits the proliferative response of D10 to IL-1

Table III. Proliferation of the Cloned Helper T Cell Line D10 is an Interleukin 4 not an Interleukin 2 Mediated Process.

D10[1]	3D3[2]	IL-1[3]	anti-IL-2[4]	anti-IL-4[5]	Response[6] Expt#1	2	3
+	-	-	-	-	122	943	2711
+	+	-	-	-	871	1704	8636
+	-	+	-	-	1049	3672	7331
+	+	+	-	-	14643	59548	49040
+	+	+	+	-	12814	57850	47100
+	+	+	-	+	3165	6034	23762

[1] 2×10^4 cells/well.
[2] 20ng/ml of 3D3.
[3] 3% P388D1 IL-1 rich supernatant.
[4] S4B6 (anti-IL-2) at 1:4 culture supernatant.
[5] 11B11 (anti-IL-4) at 1:200 SAS cut ascites.
[6] Mean CPM of tritiated thymidine incorporation of triplicate cultures, pulsed for the last 4 hours of a 48 hour assay.

Figure:11

BSF-1/IL-4 (dilution of stock solution)

Figure 11: D10 cells at 20,000 cells/well were incubated in triplicate culture for forty-eight hours with varying concentrations of affinity purifird BSF-1/IL-4 in the presence or absence of recombinant murine IL-1 (RIL-1) and in the presence of RIL-1 and 11B11. 11B11 is a monoclonal antibody with specificity against BSF-1/IL-4 Proliferation was measured by pulsing for the last four hours of the assay with 1μCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.

Figure: 12
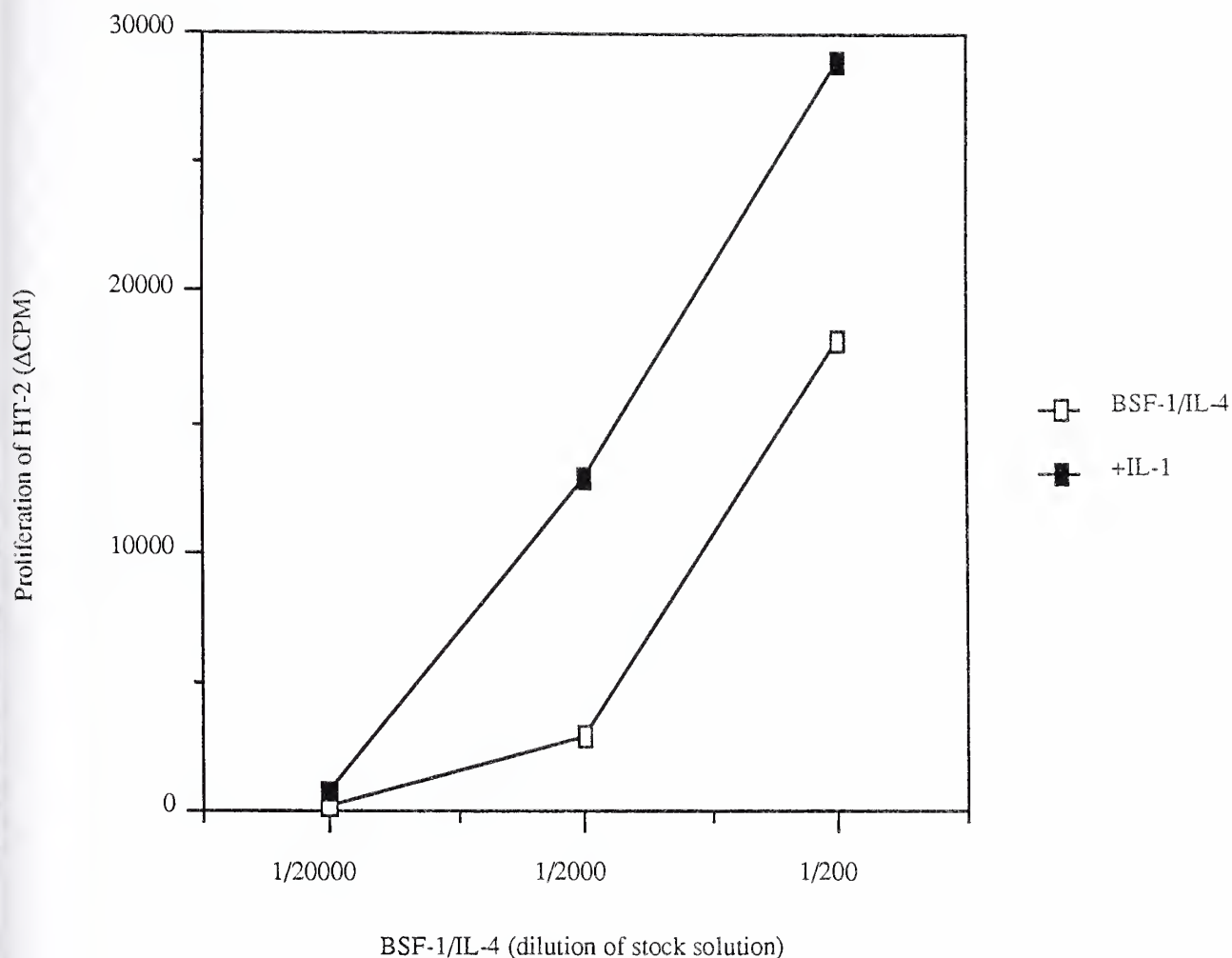
Figure 12: HT-2 cells at 10,000 cells/well were incubated in
triplicate culture for twenty-four hours with varying
concentrations of affinity purified BSF-1/IL-4 in the presence
or absence of an IL-1 rich P388D.1 supernatant. Proliferation
was measured by pulsing for the last four hours of the assay
with 1μCi/well of tritiated thymidine. Mean ±SD (<20%,
omitted from figure) was calculated for each triplicate culture.
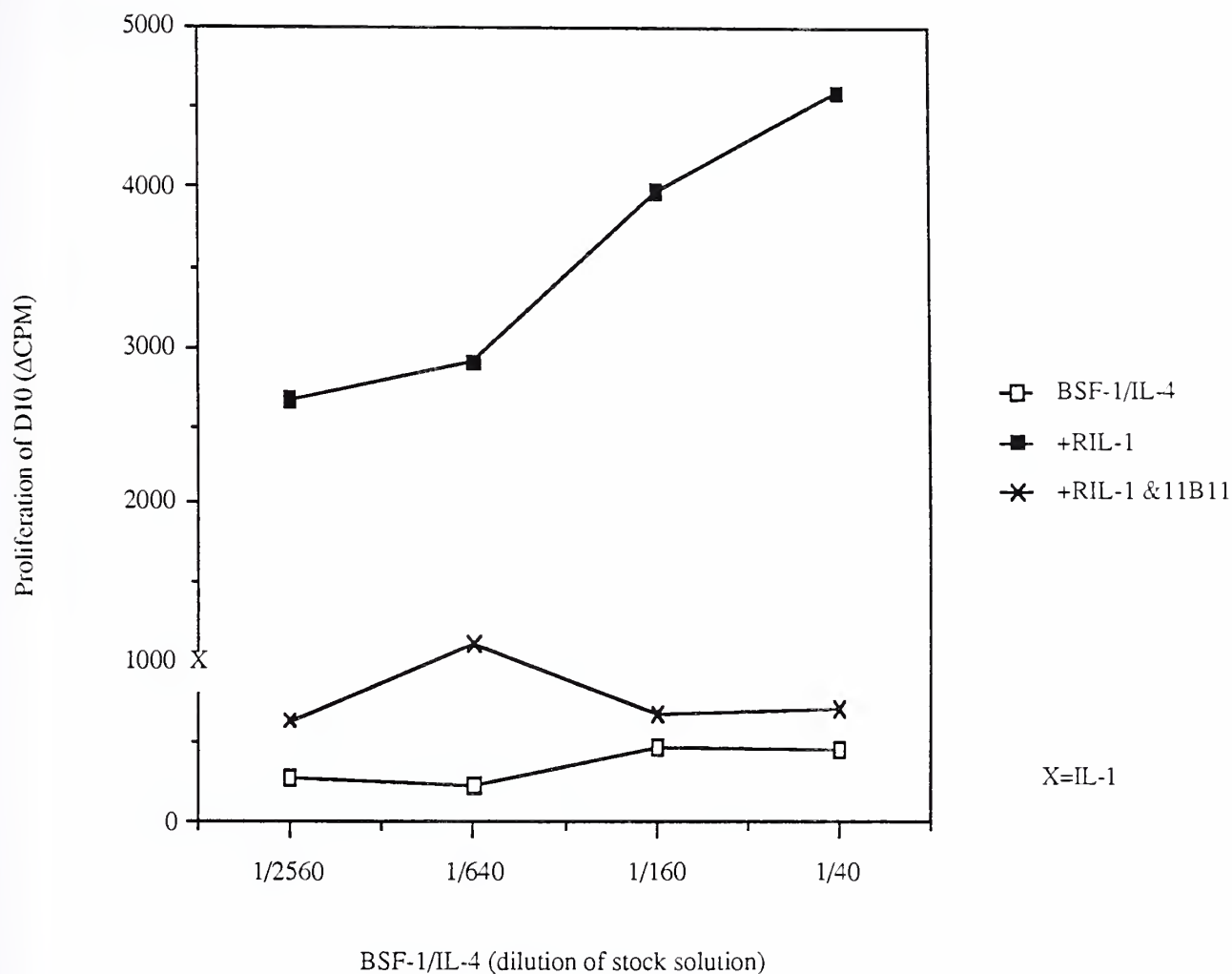
Figure:13
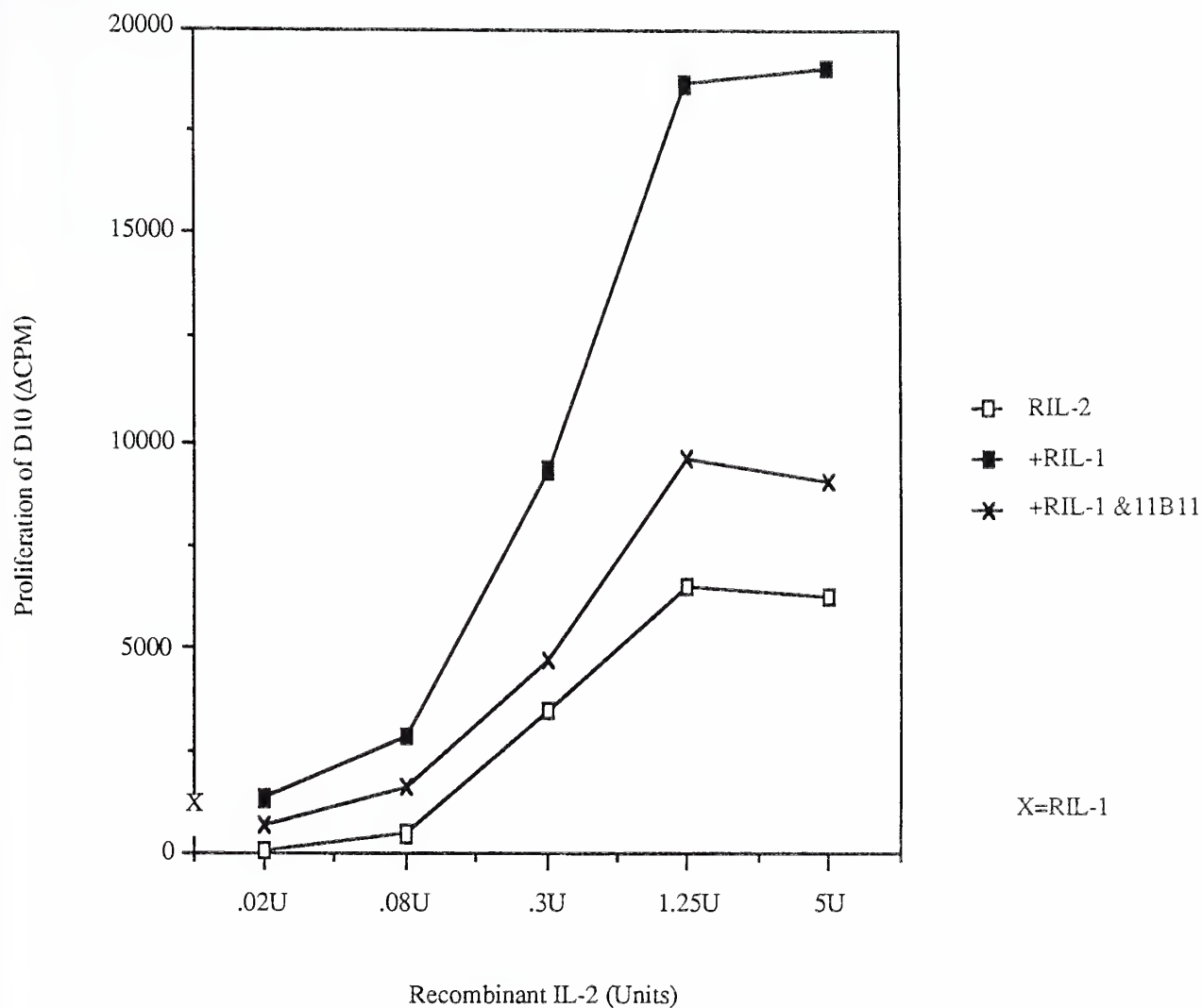
Figure 13: D10 cells at 20,000 cells/well were incubated
in triplicate culture for forty-eight hours with varying
concentrations of murine recombinant IL-2 (RIL-2) in
the presence or absence of recombinant murine IL-1
(RIL-1) and in the presence of RIL-1 and 11B11.
11B11 is a monoclonal antibody with specificity against
BSF-1/IL-4 Proliferation was measured by pulsing for
the last four hours of the assay with 1µCi/well of
tritiated thymidine. Mean ±SD (<20%, omitted from
figure) was calculated for each triplicate culture.

plus IL-2. A possible explanation for this observation is that IL-2 causes D10 to secrete IL-4 which in turn accounts for part of the proliferative response. This is similar to the observation of Howard et al.[93] who noted a similar finding with their antigen reactive T cell clones. Interleukin 1 does not augment the response of HT-2 cells to IL-2 (Fig. 14). A likely explanation for this phenomenon is the fact that HT-2 cells constitutively express a very high number of high affinity IL-2 receptors (R. Robb, personal communication).

Figure: 14

Figure 14: HT-2 cells at 10,000 cells/well were incubated in triplicate culture for twenty-four hours with varying concentrations of recombinant IL-2 in the presence or absence of an IL-1 rich P388D.1 supernatant. Proliferation was measured by pulsing for the last four hours of the assay with 1μCi/well of tritiated thymidine. Mean ±SD (<20%, omitted from figure) was calculated for each triplicate culture.
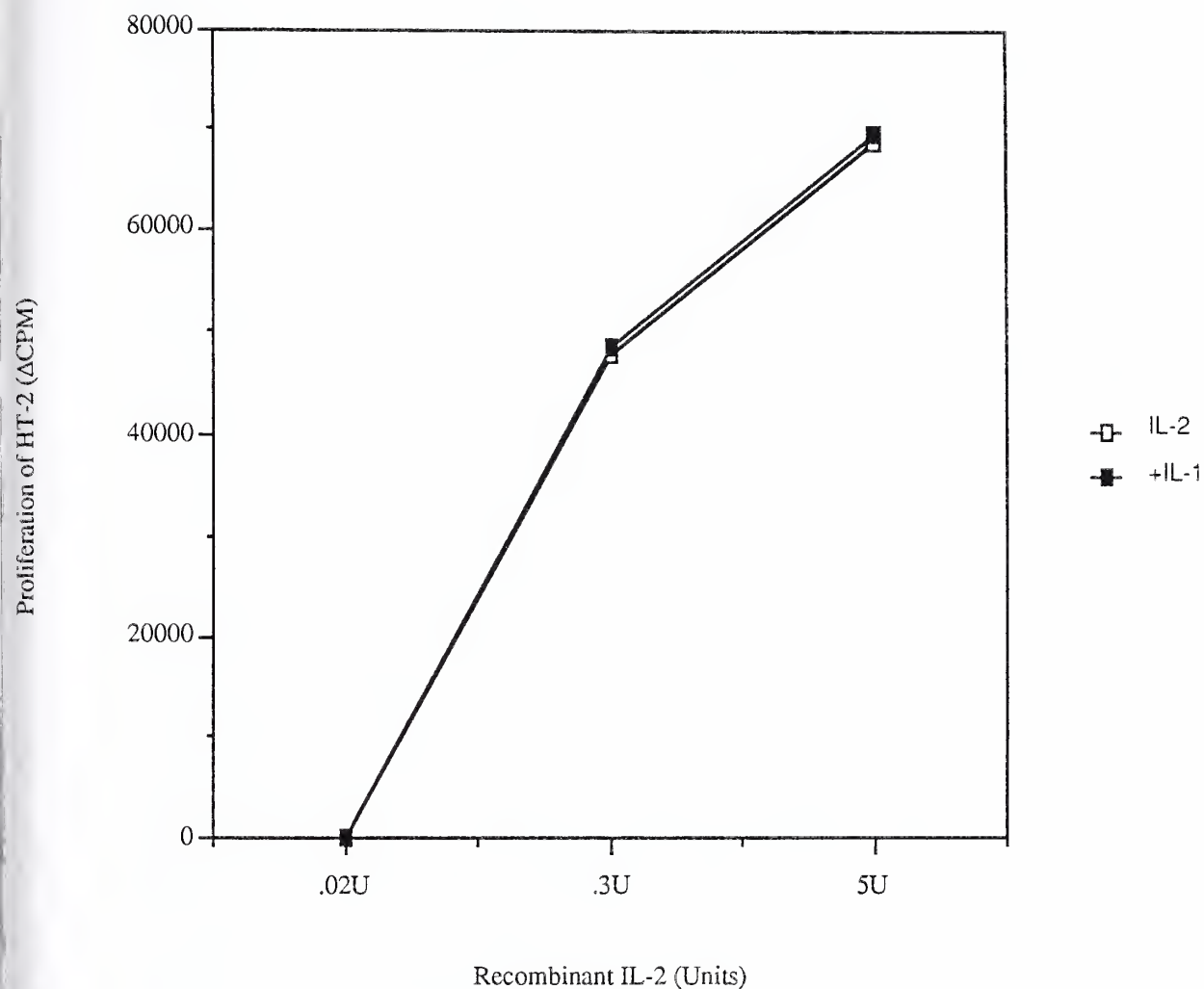
Discussion

The cloned murine T helper cell line D10.G4.1 was initially described by Kaye et al. [34]. They noted that stimulation with an anti-receptor monoclonal antibody resulted in secretion of lymphokines including a factor with TCGF activity. Further characterization of this factor revealed that it caused the TCGF dependent line HT-2 to proliferate. In addition, the anti-IL-2 antibody prepared by Gillis and Henney[157] was shown to inhibit the proliferation of D10 in response to 3D3 and IL-1[27]. Taken together these observations provided convincing evidence that stimulation of D10 resulted in the release of IL-2. However, evidence to the contrary soon followed. Using an anti-IL-2 antibody prepared by Smith et al.[158] it was observed that the TCGF activity of D10 supernatant could not be inhibited while that of AOFS supernatant could (JBH, unpublished observations). This finding combined with those presented here that the TCGF activity in D10 supernatant can be blocked by monoclonal antibodies to IL-4 but not to IL-2 strongly suggests that the TCGF activity in D10 supernatant is due to IL-4 and not IL-2. We are currently using probes for IL-2mRNA and IL-4mRNA to definitively determine that D10 does not produce IL-2. These findings also suggest that the antibody produced by Gillis and Henney might not have been an anti-IL-2 antibody but in actuality may have been an anti-IL-4 antibody.

The paradox of why D10 to fails to proliferate in response to high levels of TCGF was also examined. The finding that a diluted D10 supernatant caused a greater proliferative response than a more concentrated one suggested one reason that D10 did not proliferate in response to high levels of TCGF was due to the presence of an inhibitor. It appeared that the inhibitory effect of this factor could be overcome by adding IL-1. The observations that D10 supernatant inhibited the response of other cell lines to IL-2 further

supported this notion. Partial purification of this factor by Horowitz et al.[159] revealed that it was heat and acid stable and had a molecular weight of approximately 12,000 daltons. It was also noted that purification of Inhibitor significantly increased its inhibitory action. As shown here Inhibitor is not IFN-γ nor is it IL-4. Given that D10 does not secrete lymphotoxin[160], Inhibitor is not lymphotoxin. The observation that IL-1 can reverse can the action of Inhibitor makes it highly unlikely the Inhibitor is the so-called "inhibitor of DNA synthesis" described by Namba and Waksman[161]. They found the action of their factor completely irreversible by thirty hours while in the presence of IL-1 and D10 supernatant D10 cells are synthesizing DNA, as measured by incorporation of tritiated-thymidine, forty-eight hours after initial exposure to Inhibitor.

A number of other investigator have reported the existence of factors inhibiting IL-2[162,163,164,165]. Hardt et al. reported the presence of an IL-2 inhibitor in the sera of normal mice but not in the sera of athymic mice[162]. They found this inhibitor to be neither antigen specific nor MHC restricted. In addition, they noted of molecular weight of about 50,000 daltons. Although the biologic activity of this IL-2 inhibitor is similar to Inhibitor it differs in that in it appeared to be produced by Lyt-23+ cells and it had an apparent molecular weight of 50,000 daltons.

Kramer and Koszinowski found that Con A activated spleen cell produced IL-2 and factor(s) that inhibited proliferation of T cells to alloantigen and the generation of cytotoxic T cells from their precursors[163]. Their factor also inhibited the release of IL-2 from T cells; however, it did not affect the IL-2 mediated growth of T cell blasts. Given that Inhibitor activity is measured by its ability to suppress proliferation due to IL-2 it is most unlikely that their factor and Inhibitor are the same.

A factor that was secreted by cultured glioblastoma cells and was capable of inhibiting the response of cloned T cells line to IL-2 in addition to inhibiting the proliferation of a neuroblastic cell line whose growth was not IL-2 dependent was reported by Fontana et al.[164]. They also noted a molecular weight of 97,000 daltons. The fact that this factor inhibited the proliferation of a cell line that was not IL-2 dependent suggests that this factor may be a non-specific growth suppressor. This combined with its high molecular weight argue against this molecule and Inhibitor being the same.

Lastly, Honda et al. have recently described an IL-2 inhibitor produced by Con A stimulated spleen cells[165]. They found that it was produced by Ly-1+ and Ly-2+ T cells. Additionally, they noted that it had a molecular weight of 10,000 to 12,000 daltons and that no inhibitor activity could be detected in the supernatants of Con A stimulated spleen cells until day three. Although Inhibitor and this factor have similar molecular weights they differ in that differ markedly in their release kinetics; Inhibitor secretion occurs shortly (within twenty-four hours) after stimulation of D10 while Honda et al. find no detectable inhibitor activity until day three post stimulation. However, it is possible that the differences in release kinetics are due to the fact that D10 is not a resting T cell. Whether these two factors are the same or different remains unknown.

The existence of an inhibitor to IL-2 is not surprising In fact it would help explain why *in vivo* IL-2 is only effective in close proximity to the secreting cells. Furthermore, it seems logical that a system, like the immune system, which depends upon rapid and specific activation of only a very limited number of cells would secrete factors that activate and inhibit other immune cells. Liao et al. recent description an inhibitor of IL-1 in the urine of febrile patients would support this contention[166]. The finding that D10 does not secrete IL-2 but IL-4

raises the question of what role Inhibitor plays in IL-4 mediated proliferation. This question is currently being investigated.

The finding that the IL-4 dependent cell line D10 requires IL-1 in order to proliferate in response to IL-4 although not directly made by Kaye et al. could have been implied from their previously published reports[27,43]. As previously noted, Kaye et al. reported that when D10 cells are stimulated with 3D3 and IL-1 there is at least a ten-fold increase in 7D4 binding protein expressed on the cell surface as analyzed by FACS. Using radiolabeled IL-2 it was determined that IL-1 results in a two-fold increase in high affinity IL-2 receptors (R. Robb and JBH, unpublished observations). Thus, IL-1 has definitively been shown to increase the number of high affinity IL-2 binding sites on D10. Given that D10 does not secrete IL-2 the question that naturally arises is: what function do the high affinity IL-2 receptors have on D10? The observation that stimulation of D10 with IL-2 results in secretion of IL-4 in addition to proliferation provides at least a partial answer to that question. Although the relative contributions of IL-2 and IL-4 on D10 proliferation is difficult to determine, it appears that IL-2 results in at least some degree of proliferation independent of IL-4.

The observations that IL-4 mediated proliferation is augmented by or requires IL-1 differs from those made by Hu-Li et al.[116]. Using a heterogeneous population of resting T cells from peripheral lymph nodes they found that PMA along with IL-4 were comitogenic for resting T cells. They noted that neither PMA nor IL-4 resulted in the proliferation of resting T cells, that IL-1 did not synergize with either PMA or IL-4 to result in T cell proliferation, and that T cells precultured with either PMA or IL-4 failed to respond to subsequent culture with PMA or IL-4 but did respond subsequent culture with both PMA and IL-4. Although these observations appear incompatible with the aforementioned finding that IL-1 is required for or augments IL-4 mediated proliferation they may

not actually be so. The finding that IL-1 and IL-4 are not comitogenic for resting T cells is not surprising. Currently there are no published reports suggesting that resting T cells express functional receptors for IL-1. Because of its ability to replace accessory cells in Con A induced IL-2 secretion and IL-2 receptor expression by L3T4^{+} T cells, PMA at 10ng/ml is considered by some investigators to be functionally equivalent to IL-1[167]. If this assumption is correct, it is possible that the reason why IL-4 and PMA are synergistic for resting T cells is that PMA is acting as an IL-1 analog, and thus increasing IL-4 receptor expression. The observation by Hu-Li et al. that resting T cells pretreated with PMA fail to proliferate when subsequently treated with IL-4 is also contrary to our findings. Given that they pretreated their T cells with 1ng/ml of PMA and used a ten-fold higher concentration of PMA in their IL-4 plus PMA costimulation experiments, it is possible that at a concentration of 1ng/ml PMA does not act as an IL-1 analog. Taking all of the above into consideration, the findings of Hu-Li et al. are not necessarily inconsistent with ours. We are currently involved in competitive binding experiments to definitively prove that IL-1 induces the expression of IL-4 receptors.

The finding that IL-1 and IL-4 are synergistic on HT-2 cells is most intriguing. The HT-2 cell line is unlike most other cloned murine T helper cell lines in that it is strictly dependent on TCGF and does not require antigen for its growth[156]. Furthermore, this cell line constitutively expresses a very high number (>10,000) of high affinity receptors (R. Robb personal communication). The ability of the cell line to respond to IL-1 and IL-4 argues that HT-2 cells maintain at least some degree of control over lymphokine receptor expression and that observations deduced from this cell may also be relevant to other cell lines.

The findings that D10 cells could be pretreated with IL-1 and then be subsequently stimulated to maximal proliferation with only 3D3 suggests that "resting" D10 under the currently employed cell culturing conditions expresses IL-1 receptors. Previous experiments have demonstrated that "resting" D10 expresses about 1,000 high affinity IL-2 receptors (R. Robb and JBH, unpublished observations). Additionally, it appears that "resting" D10 does not express significant numbers of IL-4 receptors; however, stimulation with IL-1 in the presence of IL-1 receptor most probably induces IL-4 receptor expression. Thus it appears that D10 is unlike resting T cells in that it expresses both IL-1 and IL-2 receptors. These findings make the D10 cell line ideal for studying lymphokines given the presence of lymphokine receptors on its plasma membrane.

In summary, it appears that there are at least two possible explanations of why D10 fails to proliferate in response to 3D3. The first explanation is that in addition to IL-4 D10 produces an inhibitor of IL-2 and possibly IL-4. Another reason for the failure of D10 to grow in response to apparently high levels of IL-4 may reside in the observation that D10 does not express significant numbers of IL-4 receptors. The relative contribution each plays has yet to be definitively determined; however, the initial evidence suggests that the latter may be more important.

REFERENCES

[1] Silverstein, A. M. 1984. The History of Immunology. In: **Fundamental. Immunology** ed. Paul, W. E. (Raven Press) 23-40.

[2] Mish, F. C. (ed.) 1986. **Webster's Ninth New Collegiate Dictionary**. (Merriam-Webster).

[3] Silverstein, A. M. 1979. Cellular Versus Humoral Immunity: Determinants and Consequences of an Epic 19th Century Battle. **Cell. Immunol.**, **48**: 208.

[4] Lawton, A. R. and Cooper, M. D. 1983. Immune Deficiency Diseases. In: **Harrison's Principles of Internal Medicine**. eds. Peterdorf, R. G. et al. (McGraw-Hill Book Co.) 354-62.

[5] Paul, W. E. 1984. The Immune System. In: **Fundamental Immunology**. ed. Paul, W. E. (Raven Press) 3-22.

[6] Shevach, E. M. 1984. Macrophages and Other Accessory Cells. In: **Fundamental Immunology**. ed. Paul, W. E. (Raven Press) 71-107.

[7] Uhr, J. and Weissmann, G. 1965. Intracellular Distribution and Degradation of Bacteriophage in Mammalian Tissue. **J. Immunol. 94**: 544.

[8] Unanue, E. R. and Cerottini, J. C. 1970. The Immunogenicity of Antigen Bound to the Plasma Membrane of Macrophages. **J. Exp. Med. 131**:711.

[9] Rosenthal, A. S. and Shevach, E. M. 1973. Function of Macrophages in Antigen Recognition by Guinea Pig T Lymphocytes. I Requirements for Histocompatible Macrophages and Lymphocytes. **J. Exp. Med. 138**: 1194.

[10] Corradin, G., Etlinger, H. M., and Chiller, J. C. 1977. Lymphocyte Specificity to Protein Antigens I. Characterization of the Antigen-Induced *in Vitro* T Cell-Dependent Proliferative Response with Lymph Node Cells from Primed Mice. **J. Immunol. 119**: 1048.

[11] Kappler, J. W. , Skidmore, B., White, J., and Marrack, P. 1981. Antigen Inducible, H-2 Restricted Interleukin-2 Producing T Cell Hybridomas. Lack of Independent Antigen and H-2 Recognition. **J. Exp. Med. 153**: 1198.

[12] Unanue, E. R. 1984. Antigen-presenting Function of the Macrophage. **Ann. Rev. Immunol. 2**: 395.

[13] Steinman, R. M. and Witmer, M. D. 1978. Lymphoid Dendritic Cells are Potent Stimulators of the Primary Mixed Leukocyte Reaction in Mice. **Proc. Natl. Acad. Sci. USA 75**: 5132.

[14]Stingl, G., Katz, S. I., Clement, L., Green, I., and Shevach, E. M. 1978 Immunologic Functions of Ia-Bearing Epidermal Langerhan Cells. **J. Immunol. 121**: 2005.

[15]Rogoff, T. M. and Lipsky, P. E. 1979. Characterization of Isolated Guinea Pig Kupffer Cells: Accessory Cell Function in Mitogen-Induced T Lymphocyte Activation. **J. Immunol. 123**: 1920.

[16]Lipscomb, M. F., Toews, G. B., Lyons, C. R., and Uhr, J. W. 1981. Antigen Presentation by Guinea Pig Alveolar Macrophages. **J. Immunol. 126**: 286.

[17]Chestnut, R. W. and Grey, H. M. 1981. 1981. Studies on the Capacity of B Cells to Serve as Antigen-Presenting Cells. **J. Immunol. 126**: 1075.

[18]Chestnut, R. W., Colon, S. M., and Grey, H. M. 1982. Antigen Presentation by Normal B Cells, B Cell Tumors, and Macrophages: Functional and Biochemical Comparison. **J. Immunol. 128**: 1764.

[19]Cantor, H. 1984. T Lymphocytes. In: **Fundamental Immunology**. ed. Paul, W. E. (Raven Press) 57-69.

[20]Cantor, H. and Boyse, E. A. 1975. Functional Subclasses of T Lymphocytes Bearing Different Ly Antigens. I The Generation of Functionally Distinct T Cell Subclasses is a Differentiative Process Independent of Antigen. **J. Exp. Med. 141**: 1376.

[21]Cantor, H. and Boyse, E. A. 1975. Functional Subclasses of T Lymphocytes Bearing Different Ly Antigens. II Cooperation Between Subclasses of Ly+ cells in the Generation of Killer Activity. **J. Exp. Med. 141**: 1390.

[22]Rao, A., Allard, W. J., Hogan, P. G., et al. 1983. Alloreative T-Cell Clones. Ly Phenotypes Predict Both Function and Specificity for Major Histocompatibility Complex Products. **Immunogenetics 17**: 147.

[23]Kaye, J. and Janeway Jr., C. A. 1984. The $\alpha-$ and $\beta-$Subunits of a Murine T Cell Antigen/Ia Receptor have a Molecular Weight of 31,000 in the Absence of N-linked Glycosylation. **J. Immunol. 133**: 2291.

[24]Kaye, J. and Janeway Jr., C. A. 1984. The Fab Fragment of a Directly Activating Monoclonal Antibody that Precipitates a Disulfide-Linked Hetrodimer from a Helper T Cell Clone Blocks Activation by Either Allogenic Ia or Antigen and Self-Ia. **J. Exp. Med. 159**: 1397.

[25]Smith, K. A., Gilbride, K. J., and Favata, M. F. 1980. Lymphocyte Activating Factors Promotes T-Cell Growth Factor Production by Cloned Murine Lymphoma Cell. **Nature 287**: 853.

[26]Gillis, S. and Mizel, S. B. 1981. T-Cell Lymphoma Model for the Analysis of Interleukin-1-Mediated T-Cell Activation. **Proc. Natl. Acad. Sci. USA 78**: 1133.

[27]Kaye, J. Gillis, S., Mizel, S. B., et al. 1984. Growth of a Cloned Helper T Cell Line by a Monoclonal Antibody Specific for the Antigen Receptor. Interleukin 1 is Required for the Expression of Receptors for Interleukin 2. **J. Immunol. 133**: 1339.

[28]Malek, T. R, Schmidt, J. A., and Shevach, E. M., 1985. The Murine IL 2 Receptor. III Cellular Requirements for the Induction of IL 2 Receptor Expression on T Cell Subpopulations. **J. Immunol. 134**: 2405.

[29]Ashman, R. F. 1984. Lymphocyte Activation. In: **Fundamental Immunology** ed. Paul, W. E. (Raven Press) 267-300.

[30]Akerman, K. E. O. and Andersson, L. C. 1984. Direct Mitogenic Effect of Ionophore A23187 on Isolated Human T Helper Lymphocytes. **Eur. J. Immunol. 14**: 286.

[31]Infante, A. J., Infante, P. D., Gillis, S., and Fathman, C. G. 1982. Definition of T Cell Idiotypes Using Anti-idiotypic Antisera Produced by Immunization with T Cell Clones. **J. Exp. Med.155**:1100.

[32]White,J., Haskins,K. M. Marrack,P., and Kappler, J. 1983. Use of I Region-restricted, Antigen-specific T Cell Hybridomas to Produce Idiotypically Specific Anti-receptor Antibodies. **J. Immunol. 130**: 1033.

[33]Meuer, S.C., Fitzgerald, K. A., Hussey,R. E., et al. 1983. Clonotypic Structures Involved in Antigen-specific Human T Cell Function. **J. Exp. Med. 157**: 705.

[34]Kaye, J., Porcelli, S., Tite, J., et al. 1983. Both a Monoclonal Antibody and Antisera Specific for Determinants Unique to Individual Clone Helper T Cell Lines Can Substitute for Antigen and Antigen-Presenting Cells in the Activation of T Cells. **J. Exp. Med. 158**: 836.

[35]Sharon, N. and Lis, H. 1972. Lectins: Cell Agglutinating and Sugar-specific Proteins. **Science 177**: 949.

[36]Wedner,H. J. and Parker, C. W. 1976. Lymphocyte Activation. In: **Progress in Allergy Vol. 20** eds. Kallos, P., et al.(S Karger AG) 195-300.

[37]Tsien, R. Y., Pozzan, T., and Rink, T. J. 1982. T-Cell Mitogens Cause Early Changes in Cytoplasmic Free Ca^{2+} and Membrane Potential in Lymphocytes. **Nature 295**: 68.

[38]Luckasen, J. R., White, J. G., and Kersey, C. W. 1974. Mitogenic Properties of a Calcium Ionophore, A23187. **Proc. Natl. Acad. Sci. USA 71**: 5088.

[39]Waldmann, H. 1977. Conditions Determining the Generation and Expression of T Helper Cells. **Immunol. Rev. 35**: 121.

[40]Tada, T., Toshitada, T., Okumura, K., et al. 1978. Two Distinct Types of Helper T Cells Involved in the Secondary Antibody Response: Independent and Synergistic Effects of Ia⁻ and Ia⁺ Helper Cells. **J. Exp. Med. 147**: 446.

[41]Swierkosz, J. E., Marrack, P., and Kappler, J. W. 1979. Functional Analysis of T Cells Expressing IA Antigens. I Demonstration of Helper T-Cell Heterogeneity. **J. Exp. Med. 150**: 1293.

[42]Imperiale, M. J., Faherty, D. A., Sproviero, J. F., and Zauderer, M. 1982. Functionally Distinct Helper T Cells Enriched Under Different Culture Conditions Cooperate with Different B Cells. **J. Immunol. 129**: 1843.

[43]Kim, J., Woods, A., Becker-Dunn, E., and Bottomly, K. 1985. Distinct Functional Phenotypes of Cloned Ia-Restricted Helper T Cells. **J. Exp. Med. 162**: 188.

[44]Mosmann, T. R., Cherwinski, H., Bond, M. W., et al. 1986. Two Types of Murine Helper T Cell Clone. I Definition According to Profiles of Lymphokine Activities and Secreted Proteins. **J. Immunol. 136**: 2348.

[45]Bottomly, K., Jones, B., Kaye, J., and Jones, F. 1983. Subpopulations of B Cells Distinguished by Cell Surface Expression of Ia Antigens. Correlation of Ia and Idiotype During Activation by Cloned Ia-Restricted T Cells. **J. Exp. Med. 158**: 265.

[46]Killar, L., MacDonald, G., West, J., Woods, A., and Bottomly, K. 1987. Cloned, Ia-Restricted T Cells That Do Not Produce Interleukin 4 (IL-4)/ B Cell Stimulatory Factor 1 (BSF-1) Fail To Help Antigen-Specific B Cells. **J Immunol**. in press.

[47]Kappler, J. and Marrack, P. 1986. Lymphokines. In: **Handbook of Experimental Immunology**. eds. Weir, D. M. et al. (Blackwell Scientific Publications) 59.1-59.10.

[48]Smith, K. A. 1984. Lymphokine Regulation of T Cell and B Cell Function. In: **Fundamental Immunology**. ed. Paul, W. E. (Raven Press) 559-576.

[49]Rabin, E. M., Ohara, J., and Paul, W. E. 1985. B-Cell Stimulatory Factor 1 Activates Resting B Cells. **Proc. Natl. Acad. Sci. USA 82**: 2935.

[50]Fernandez-Botran, R., Krammer, P. H., Diamantstein, T., Uhr, J. W., and Vitetta, E. S. 1986. B Cell-Stimulatory Factor 1 (BSF-1) Promotes Growth of Helper T Cell Lines. **J. Exp. Med. 164**: 580.

[51]Robb, R. J., Munick, A., and Smith, K. A. 1981. T Cell Growth Factor Receptors. Quantitation, Specificity, and Biological Relevance. **J. Exp. Med. 154**: 1455.

[52]Beeson, P. B. 1948. Temperature-Elevating Effect of a Substance Obtained from Polymorphonuclear Leukocytes. **J. Clin. Invest. 27**: 524.

[53]Dinarello, C. A. 1984. Interleukin-1. **Rev. Infect. Dis. 6**: 51.

[54]Gery, I., Gershon, R. K., and Waksman, B. H. 1971. Potentiation of Cultured Mouse Thymocyte Responses by Factors Released by Peripheral Leucocytes. **J. Immunol. 107**: 1778.

[55]Gery, I., Gershon, R. K., and Waksman, B. H. 1972. Potentiation of the T-Lymphocyte Response to Mitogens. I The Responding Cell. **J. Exp. Med. 136**: 128.

[56]Gery, I. and Waksman, B. H. 1972. Potentiation of the T-Lymphocyte Response to Mitogens. II The Cellular Source of Potentiating Mediator(s). **J. Exp. Med. 136**: 143.

[57]Rosenwasser, L. J., Dinarello, C. A. and Rosenthal, A. S. 1979. Adherent Cell Function in Murine T-Lymphocyte Antigen Recognition. IV. Enhancement of Murine T-Cell Antigen Recognition by Human Leukocytic Pyrogen. **J. Exp. Med. 150**: 709.

[58]Rosenwasser, L. J. and Dinarello, C. A. 1981. Ability of Human Leukocytic Pyrogen to Enhance Phytohemagglutinin Induced Murine Thymocyte Proliferation. **Cell Immunol. 63**: 134.

[59]Blyden, G. and Handschumacher, R. E. 1977. Purification and Properties of Human Lymphocyte Activating Factor (LAF). **J. Immunol. 118**: 1631.

[60]Mizel, S. B. and Mizel, D. 1981. Purification to Apparent Homogeneity of Murine Interleukin 1. **J. Immunol. 126**: 834.

[61]Lomedico, P. T., Gubler, U., Hellmann, C. P., et al. 1984. Cloning and Expression of Murine Interleukin-1 cDNA in *Escherichia coli*. **Nature 312**: 458.

[62]March, C. J., Mosley, B., Larsen, A., et al. 1985. Cloning, Sequence and Expression of Two Distinct Human Interleukin-1 Complementary DNAs. **Nature 315**: 641.

[63]DeChiara, T. M., Young, D., Semionow, R., et al. 1986. Structure-Function Analysis of Murine Interleukin 1: Biologically Active Polypeptides are at Least 127 Amino Acids Long and are Derived From the Carboxyl Terminus of a 270-Amino Acid Precursor. **Proc. Natl. Acad. Sci. USA 83**: 8303.

[64]Dower, S. K., Kronheim, S. R., March, C. J., et al. 1985. Detection and Characterization of High Affinity Plasma Membrane Receptors For Human Interleukin 1. **J. Exp. Med 162**: 501.

[65]Dower, S. K., Call, S. M., Gillis, S., and Urdal, D. L. 1986. Similarity Between the Interleukin 1 Receptor on a Murine T-Lymphoma Cell Line and a Murine Fibroblast Cell Line. **Proc. Natl. Acad. Sci. USA 83**: 1060.

[66]Bird, T. A. and Saklatvala, J. 1986. Identification of a Common Class of High Affinity Receptors for Both Types of Porcine Interleukin-1 on Connective Tissue Cells. **Nature 324**: 263.

[67]Dower, S. K., Kronheim, S. R., Hopp, T. P., et al. 1986. The Cell Surface Receptors for Interleukin-1α and Interleukin-1β are Identical. **Nature 324**: 266.

[68]Kilian, P. L., Kaffka, K. L., Stern, A. S., et al. 1986. Interleukin 1α and Interleukin 1β Bind to the Same Receptor on T Cells. **J. Immunol. 136**: 4509.

[69]Dinarello, C. A. 1984. Interleukin-1 and the Pathogenesis of the Acute-Phase Response. **N. Engl. J. Med. 311**: 1413.

[70]Lipsky, P. E., Thompson, P. A., Rosenwasser, L. J., and Dinarello, C. A. 1983. The Role of Interleukin 1 in Human B Cell Activation: Inhibition of B Cell Proliferation and the Generation of Immunoglobulin-Secreting Cells by an Antibody Against Human Leukocytic Pyrogen. **J. Immunol. 130**: 2708.

[71]Howard, M., Nakanishi, K., and Paul, W. E. 1984. B Cell Growth and Differentiation Factors. **Immunol. Rev. 78**: 185.

[72]Morgan, D. A., Ruscetti, F. W., and Gallo, R. C. 1976. Selective *in Vitro* Growth of T-Lymphocytes from Normal Human Bone Marrow. **Science 193**: 1007

[73]Ruscetti, F. W., Morgan, D. A.,and Gallo, R. C. 1977. Functional and Morphologic Characterization of Human T Cells Continuously Grown *in Vitro*. **J. Immunol. 119**: 131.

[74]Gillis, S. and Smith, K. A. 1977. Long Term Culture of Tumour-Specific Cytotoxic T Cells. **Nature 268**: 154.

[75]Gillis, S., Ferm, M. M., Ou, W., and Smith, K. A. 1978. T Cell Growth Factor: Parameters of Production and a Quantitative Microassay for Activity. **J. Immunol. 120**: 2027.

[76]Gillis, S.,Smith, K. A., and Watson, J. 1980. Biochemical Characterization of Lymphocyte Regulatory Molecules. II Purification of a Class of Rat and Human Lymphokines. **J. Immunol. 124**: 1954.

[77]Watson, J., Gillis, S., Marbrook, J., et al. 1979. Biochemical and Biological Characterization of Lymphocyte Regulatory Molecules. I Purification of a Class of Murine Lymphokines. **J. Exp. Med. 150**: 849.

[78]Taniguchi, T., Matsui, H., Fujita, T., et al. 1983. Structure and Expression of a Cloned cDNA for Human Interleukin-2. **Nature 302**: 305.

[79]Robb, R. J. 1984. Interleukin 2: The Molecule and Its Function. **Immunol. Today 5**: 203.

[80]Smith, K. A., Gillis, S., Baker, P. E., et al. 1979. T-Cell Growth Factor-Mediated T-Cell Proliferation. **Ann. N. Y. Acad. Sci. 332**: 423.

[81]Smith, K. A. 1980. T-Cell Growth Factor. **Immunol. Rev. 51**: 337.

[82]Leonard, W. J., Depper, J. M., Uchiyama, T., et al. 1982. A Monoclonal Antibody that Appears to Recognize the Receptor for Human T-Cell Growth Factor; Partial Characterization of the Receptor. **Nature 300**: 267.

[83]Malek, T. R., Robb, R. J., and Shevach, E. M. 1983. Identification and Initial Characterization of a Rat Monoclonal Antibody Reactive with the Murine Interleukin 2 Receptor-Ligand Complex. **Proc. Natl. Acad. Sci. USA 80**: 5694.

[84]Osawa, H. and Diamantstein, T. 1984. A Rat Monoclonal Antibody that Binds Specifically to Mouse T Lymphoblasts and Inhibits IL-2 Receptor Functions. A Putative Anti-IL 2 Receptor Antibody. **J. Immunol. 132**: 2445.

[85]Osawa, H. and Diamantstein, T. 1983. The Characteristics of a Monoclonal Antibody that Binds Specifically to Rat Lymphoblasts and Inhibits IL2 Receptor Functions. **J. Immunol. 130**: 51.

[86]Leonard, W. J., Depper, J. M., Crabtree, G. R., et al. 1984. Molecular Cloning and Expression of cDNAs for the Human Interleukin-2 Receptor. **Nature 311**: 626.

[87]Nikaido, T., Shimizu, A., Ishida, N., et al. 1984.Molecular Cloning of cDNAs Encoding Human Interleukin-2 Receptor. **Nature 311**: 631.

[88]Robb, R. J., Greene, W. C., and Rusk, C. M. 1984. Low and High Affinity Cellular Receptors for Interleukin 2. Implications for the Level of Tac Antigen. **J. Exp. Med. 160: 1126.**

[89]Robb, R. J. 1986. Conversion of Low-Affinity Interleukin 2 Receptors to a High-Affinity State Following Fusion of Cell Membranes. **Proc. Natl. Acad. Sci. USA 83**: 3992.

[90]Kondo, S., Shimizu, A., Saito, Y., et al. 1986. Molecular Basis for Two Different Affinity States of the Interleukin 2 Receptor: Affinity Conversion Model. **Proc. Natl. Acad. Sci. USA 83**: 9026.

[91]Henney, C. S., Kuribayashi, K., Kern, D. E., and Gillis, S. 1981. Interleukin-2 Augments Natural Killer Cell Activity. **Nature 291**: 335.

[92]Mond, J. J., Thompson, C., Finkelman, F. D., et al. 1985. Affinity-purified Interleukin 2 Induced Proliferation of Large But Not Small B Cells. **Proc. Natl. Acad. Sci. USA 82**: 1518.

[93]Howard, M., Matis, L., Malek, T. R., et al. 1983. Interleukin 2 Induces Antigen-Reactive T Cell Lines to Secrete BCGF-1. **J. Exp. Med. 158**: 2024.

[94]Kasahara, T., Hooks, J. J., Dougherty, S. F., and Oppenheim, J., J. 1983. Interleukin 2-Mediated Immune Interferon (IFN-γ) Production by Human T Cells and T Cell Subsets. **J. Immunol.130**: 1748.

[95]Kelso, A., Metcalf, D., and Gouch, N. M. 1986. Independent Regulation of Granulocyte-Macrophage Colony-Stimulating Factor and Multi-Lineage Colony-Stimulating Production in T Lymphocytes Clones. **J. Immunol. 136**: 1718.

[96]Corley, R. B., Dexter, C. M., and Ovnic, M. 1986. Inducible Lymphokines of T Cell Tumors. **CRC Crit. Rev. Immunol. 6**: 71.

[97]Ihle, J. N., Pepersack, L. ,and Rebar, L. 1981. Regulation of T Cell Differentiation: *in Vivo* Induction of 20α-Hydroxysteroid Dehydrogenase in Splenic Lymphocytes From Athymic Mice is Mediated by a Unique Lymphokine. **J. Immunol. 126**: 2184.

[98]Garland, J. M. 1984. Involvement of Interleukin 3 in Lymphocyte Biology and Leukemogenesis In: **Lymphokines** ed. Pick, E. (Academic Press, Inc.) 153-200.

[99]Ihle, J. N., Keller, J., Henderson, L., et al. 1982. Procedures for the Purification of Interleukin 3 to Homogeneity. **J. Immunol. 129**: 2431.

[100]Fung, M. C., Hapel, A. J., Ymer, S., et al. Molecular Cloning of cDNA for Murine Interleukin-3. **Nature 307**: 233.

[101]Yokata, T., Lee, F., Rennick, D., et al. 1984. Isolation and Characterization of a Mouse cDNA that Expresses Mast-Cell Growth-Factor Activity in Monkey Cells. **Proc. Natl. Acad. Sci. USA 81**: 1070.

[102]Howard, M., Kessler, S., Chused, T., and Paul, W. E. 1981. Long-Term Culture of Normal Mouse B Lymphocytes. **Proc. Natl. Acad. Sci. USA 78**: 5788.

[103]Howard, M., Farrar, J., Hilfiker, M., et al. 1982. Identification of a T Cell-Derived B Cell Growth Factor Distinct From Interleukin 2. **J. Exp. Med. 155**: 914.

[104]Ohara, J., Lahet, S., Inman, J., and Paul, W. E. 1985. Partial Purification of Murine B Cell Stimulatory Factor (BSF-1). **J. Immunol. 135**: 2518.

[105]Ohara, J. and Paul, W. E. 1985. Production of a Monoclonal Antibody to and Molecular Characterization of B-Cell Stimulatory Factor-1. **Nature 315**:333.

[106]Grabstein, K., Eisenman, J., Mochizuki, D., et al. 1986. Purification to Homogeneity of B Cell Stimulating Factor. **J. Exp. Med. 163**: 1405.

[107]Noma, Y., Sideras, P., Naito, T., et al. 1986. Cloning of cDNA Encoding the Murine IgG1 Induction Factor By a Novel Strategy Using SP6 Promoter. **Nature 319**: 640.

[108]Lee, F., Yokota, T., Otsuka, T., et al. 1986. Isolation and Characterization of a Mouse cDNA Clone that Expresses B-Cell Stimulatory Factor 1 Activities and T-Cell and Mast-Cell-Stimulating Activities. **Proc. Natl. Acad. Sci. USA 83**: 2061.

[109]Yokota, T., Otsuka, T., Mosmann, T., et al. Isolation and Characterization of a Human cDNA Clone, Homologous to Mouse B-Cell Stimulatory Factor 1, that Expresses B-Cell- and T-Cell-Stimulating Activities. **Proc. Natl. Acad. Sci. USA 83**: 5894.

[110]Mishra, G. C., Berton, M. C., Oliver, K. G., et al. 1986. A Monoclonal Anti-Mouse LFA-1α Antibody Mimics the Biological Effects of B Cell Stimulatory Factor-1 (BSF-1). **J. Immunol. 137**: 1590.

[111]Noelle, R., Krammer, P. H., Ohara, J., et al. 1984. Increased Expression of la Antigens on Resting B Cells: An Additional Role for B-Cell Growth Factor. **Proc. Natl. Acad. Sci. USA 81**: 6149.

[112]Vitetta, E. S., Ohara, J., Myers, C. D., et al. 1985. Serological, Biochemical, and Functional Identity of B Cell-Stimulating Factor 1 and B Cell Differentiation Factor for IgG1. **J. Exp. Med. 162**: 1726.

[113]Coffman, R. L. and Carty, J. 1986. A T Cell Activity That Enhances Polyclonal IgE Production and Its Inhibition by Interferon-γ. **J. Immunol. 136**: 949.

[114]Coffman, R. L., Ohara, J., Bond, M. W., et al. 1986. B Cell Stimulatory Factor-1 Enhances the IgE Response of Lipopolysaccharide- Activated B Cells. **J. Immunol.136**: 4538.

[115]Finkelman, F. D., Katona, I. M., Urban, Jr., J. F., et al. 1986. Suppression of *in Vivo* Polyclonal IgE Responses by Monoclonal Antibody to the Lymphokine B-Cell Stimulatory Factor 1. **Proc. Natl. Acad. Sci. USA 83**: 9675.

[116]Fernandez-Botran, R., Sanders, V. M., Oliver, K. G., et al. 1986. Interleukin 4 Mediates Autocrine Growth of Helper T Cells After Antigenic Stimulation. **Proc. Natl. Acad. Sci. USA 83**: 9689.

[117]Hu-Li, J., Shevach, E. M. , Mizuguchi, J., et al. 1987. B Cell Stimulatory Factor 1 (Interleukin 4) is a Potent Costimulant for Normal Resting T Lymphocytes. **J. Exp. Med. 165**: 157.

[118]Mosmann, T. R., Bond, M. W., Coffman, R. L., et al. 1986. T-Cell and Mast Cell Lines Respond to B-Cell Stimulatory Factor 1. **Proc. Natl. Acad. Sci. USA 83**: 5654.

[119]Lopez, A. F., Nicola, N. A., Burgess, A. W., et al. 1983. Activation of Granulocyte Cytotoxic Function by Purified Colony-Stimulating Factors. **J. Immunol. 131**: 2983.

[120]Gough, N. M., Gough, J., Metcalf, D., et al. 1984. Molecular Cloning of cDNA Encoding a Murine Haematopoietic Growth Regulator, Granulocyte-Macrophage Colony Stimulating Factor. **Nature 309**:763.

[121]Handman, E. and Burgess, A. 1979. Stimulation by Granulocyte-Marcophage Colony-Stimulating Factor of *Leishmania tropica* Killing by Marcophages. **J. Immunol. 122**: 1134.

[122]Dessein, A. J., Vadas, M. A., Nicola, N. A., et al. 1982. Enhancement of Human Blood Eosinophil Cytotoxicity by Semi-Purified Eosinophil Colony-Stimulating Factor(s). **J. Exp. Med. 156**: 90.

[123]Grabstein, K., Mochizuki, D., Kronheim, S., et al. 1986. Regulation of Antibody Production *in Vitro* by Granulocyte-Macrophage Colony Stimulating Factor. **J. Mol. Cell. Immunol. 2**:199.

[124]Grabstein, K., Urdal, D. L., Tushinski, R. J., et al. 1986. Induction of Macrophage Tumoricidal Activity by Granulocyte-Macrophage Colony-Stimulating Factor. **Science 232**: 506.

[125]Woods, A., West, J., Rasmussen, R., and Bottomly, K. 1987. Granulocyte-Macrophage Colony Stimulating Factor Produced by Cloned L3T4a+, Class II-Restricted T Cells Induces HT-2 Cells to Proliferate. Submitted for Publication.

[126]Gray, P. W., Leung, D. W., Pennica, D. et al. 1982. Expression of Human Immune Interferon cDNA in *E. coli* and Monkey Cells. **Nature** 295: 503.

[127]Stewart II, W. E., Blalock, J. E., Burke, D. C., et al. 1980. Interferon Nomenclature. **Nature 286**:110.

[128]Blalock, J. E., Georgiades, J. A., Langford, M. P., and Johnson, H. M. 1980. Purified Human Immune Interferon Has More Potent Anticellular Activity than Fibroblast or Leukocyte Interferon. **Cell. Immunol. 49**: 390.

[129]Catalona, W. J., Ratliff, T. L., and McCool, R. E. 1981. γ Interferon Induced by *S, aureus* Protein A Augments Natural Killing and ADCC. **Nature 291**:77.

[130]Wallach, D. 1983. Interferon-Induced Resistance to the killing by NK cells: A Preferential Effect of IFN-γ. **Cell. Immunol. 75**: 390.

[131]Steeg, P. S., Moore, R. N., Johnson, H. M., Oppenheim, J. J. 1982. Regulation of Murine Macrophage Ia Antigen Expression by a Lymphokine with Immune Interferon Activity. **J. Exp. Med. 156**: 1780.

[132]Zlonik, A., Shimonkevitz, R. P., Gefter, M. L., et al. 1983. Characterization of the γ–Interferon-Mediated Induction of Antigen-Presenting Ability in P388D1 Cells. **J. Immunol. 131**: 2814.

[133]Nakamura, M., Manser, T., Pearson, G. D. N., et al. 1984. Effect of IFN-γ on the Immune Response *in Vivo* and on Gene Expression *in Vitro*. **Nature 307**: 381.

[134]Groenewegen, G. Buurman, W. A., and van der Linden, C. J. 1985. Lymphokine Dependence of *in Vivo* Expression of MHC Class II Antigens by Endothelium. **Nature 316**: 361.

[135]Rabin, E. M., Mond,,J. J., Ohara, J., and Paul, W. E. 1986. Interferon-γ Inhibits the Action of B Cell Stimulatory Factor (BSF-1) on Resting B Cells. **J. Immunol.137**: 1573.

[136]Mond, J. J., Carman, J., Finkelman, F. D., and Ohara, J. 1986. Recombinant Interferon-γ Suppresses B Cell Stimulation Factor Induction of Class II MHC Determinants on B Cells. **Fed. Proc. 45**: 250.

[137]Ruddle, N. H. and Waksman, B. H. 1967. Cytotoxic Effect of Lymphocyte-Antigen Interaction in Delayed Hypersensitivity. **Science 157**: 1060.

[138]Ruddle, N. H. and Waksman, B. H. 1968. Cytotoxicity Mediated by Soluble Antigen and Lymphocytes in Delayed Hypersensitivity. I Characterization of the Phenomenon. **J. Exp. Med. 128**:1237.

[139]Walker, S. M., Lee, S. C., and Lucas, Z. J. 1976. Cytotoxic Activity of Lymphocytes. VI. Heterogeneity of Cytotoxins in Supernatants of Mitogen-Activated Lymphocytes. **J. Immunol. 116**: 807.

[140]Gray, P. W., Aggarwal, B. B., Benton, C. V. et al. 1984. Cloning and Expression of cDNA for Human Lymphotoxin, a Lymphokine with Tumour Necrosis Activity. **Nature 312**: 721.

[141]Evans, C. H. and Heinbaugh, J. A. 1981. Lymphotoxin Cytotoxicity, a Combination of Cytolytic and Cytostatic Cellular Responses. **Immunopharmacology 3:** 347.

[142]Sawada, J. and Osawa, T. 1980. Characterization of Lectin-Induced Cellular Cytotoxicity Mediated by Mouse Spleen Cells and the Role of Lymphotoxin. **Immunology 41**: 525.

[143]Wright, S. C. and Bonavida, B. 1981. Selective Lysis of NK-Sensitive Target Cells by a Soluble Mediator Released From Murine Spleen Cells and Human Peripheral Blood Lymphocytes. **J. Immunol. 126**: 1516.

[144]Green, L. M., Stern, M. L., Haviland, D. L., et al. 1985. Cytotoxic Lymphokines Produced By Cloned Human Cytotoxic T Lymphocytes. **J. Immunol. 135**: 4034.

[145]Kondo, L. L., Rosenau, W., and Wara, D. W. 1981. Role of Lymphotoxin in Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC). **J. Immunol.126**: 1131.

[146]Stone-Wolff, D. S., Yip, Y. K., Kelker,H. C., et al. 1984. Interrelationships of Human Interferon-Gamma with Lymphotoxin and Monocyte Cytotoxin. **J. Exp. Med. 159**: 828.

[147]Lee, S. H., Aggarwal, B. B., Rinderknecht, E., et al. 1984. Communications: The Synergistic Anti-Proliferative Effects of γ-Interferon and Human Lymphotoxin. **J. Immunol. 133**: 1083.

[148]Holley, R. W. 1975. Control of Growth of Mammalian Cells in Cell Culture. **Nature 258**: 487.

[149]Todaro, G. J. and De Larco, J. E. 1978. Growth Factors Produced by Sarcoma Virus-Transformed Cells. **Cancer Res. 38**: 4147.

[150]Sporn, M. B. and Roberts, A. B. 1985. Autocrine Growth Factors and Cancer. **Nature 313**: 745.

[151]Conrad, P. J. and Janeway, Jr., C. A. 1984. The Expression of I-E^d Molecules in F_1 Hybrid Mice Detected with Antigen-Specific, I-E^d-Restricted Cloned T-Cell Lines. **Immunogenetics 20**: 311.

[152]Tite, J. P. and Janeway, Jr., C. A. 1984. Antigen-Dependent Selection of B Lymphoma Cells Varying in Ia Density by Cloned Antigen-Specific L3T4a$^+$ T Cells: A Possible *in Vitro* Model for B Cell Adaptive Differentiation. **J. Mol. Cell. Immunol. 1**: 253.

[153]Tite, J. P. and Janeway, Jr., C. A. 1984. Cloned Helper T Cells Can Kill B Lymphoma Cells in the Presence of Specific Antigen: Ia Restriction and Cognate *vs.* Noncognate Interactions in Cytolysis. **Eur. J. Immunol. 14**: 878.

[154]Click, R. E., Benck, L. and Alter, B. J. 1972. Immune Responses in Vitro. I. Culture Conditions For Antibody Synthesis. **Cell. Immunol. 3**: 264.

[155]Janeway, Jr., C. A., Lerner, E. A., Conrad, P. J., and Jones, B. 1982. The Precision of Self and Non-Self Major Histocompatibility Complex Encoded Antigen Recognition by Cloned T Cells. **Behring Inst. Mitt. 70**: 200.

[156]Watson, J. 1979. Continuous Proliferation of Murine Antigen-Specific Helper T Lymphocytes in Culture. **J. Exp. Med. 150**: 1510.

[157]Gillis, S. and Henney, C. S. 1981. The Biochemical and Biological Characterization of Lymphocyte Regulatory Molecules. VI Generation of a B Cell Hybridoma Whose Antibody Product Inhibits Interleukin 2 Activity. **J. Immunol. 126**: 1978.

[158]Smith, K. A., Favata, M. F., and Oroszlan, S. 1983. Production and Characterization of Monoclonal Antibodies to Human Interleukin 2: Strategy and Tactics. **J. Immunol. 131**: 1808.

[159]Horowitz, J. B., Kaye, J., Conrad, P. J., et al. 1986. Autocrine Growth Inhibition of a Cloned Line of Helper T Cells. **Proc. Natl. Acad. Sci. USA 83**: 1886.

[160]Tite, J., Powell, M. B., and Ruddle, N. H. 1985. Protein-Antigen Specific Ia-Restricted Cytolytic T Cells : Analysis of Frequency, Target Cell Susceptibility, and Mechanism of. **J. Immunol. 135**: 25.

[161]Namba, Y. and Waksman, B. H. 1975. Regulatory Substances Produced by Lymphocytes. I. Inhibitor of DNA Synthesis in the Rat. **Inflammation 1**:5.

[162]Hardt, C., Rollinghoff, M., Pfizenmaier, K. et al. 1981. Lyt-23+ Cyclophosphamide-Sensitive T Cells Regulate the Activity of an Interleukin 2 Inhibitor *in Vivo*. **J. Exp. Med. 154**: 262.

[163]Kramer, M. and Koszinowski, U. 1982. T Cell-Specific Suppressor Factor(s) With Regulatory Influence on Interleukin 2 Production and Function. **J. Immunol. 128**: 784.

[164]Fontana, A. , Hengartner, H., de Tribolet, N., and Weber, E. 1984. Glioblastoma Cells Release Interleukin 1 and Factors Inhibiting Interleukin 2-Mediated Effects. **J. Immunol. 132**: 1837.

[165]Honda, M., Chan, C., and Shevach, E. M. 1985. Characterization and Partial Purification of a Specific Interleukin 2 Inhibitor. **J. Immunol. 135**: 1834.

[166]Liao, Z., Grimshaw, R. S., and Rosenstreich, D. L. 1984. Identification of a Specific Interleukin 1 Inhibitor in the Urine of Febrile Patients. **J. Exp. Med. 159**: 126.

[167]Malek, T. R., Schmidt, J. A., and Shevach, E. M. 1985. The Murine IL-2 Receptor. III. Cellular Requirements for the Induction of IL 2 Receptor Expression on T Cell Subpopulations. **J. Immunol. 134**: 2405.

APPENDIX

Publications Derived From this Thesis

Janeway, C. A., Jr., Bottomly, K., Horowitz, J., et al. 1985 Modes of Cell:Cell Communication in the Immune System. **J. Immunol. 135**: 739s.

Janeway, C. A., Jr., Tite, J. P., Horowitz, J., et al. 1986. Cell Interactions in the Immune System: The Role of Self Recognition in the Targeting of Nonspecific Effector Molecules by Helper T Cells. In: **Immunology and Cancer**. eds.Kripke, M. L. and Frost, P. (Univ. of Texas Press) 45-61.

Janeway, C. A., Jr., Conrad, P. J., Horowitz, J.B., et al. 1986. Steps in the Process of T Lymphocyte Activation. In: **Mediators of Immune Regulation and Immunotherapy**. eds. Singhal, S.K. and Delovitch, T.L. (Elsevier Science Publishing Co.) 31-39.

Horowitz, J. B., Kaye J., Conrad, P. J., et al. 1986. Autocrine Growth Inhibition of a Cloned Line of Helper T Cells. **Proc. Natl. Acad. Sci. USA 83**: 1886.

Jones, B., Horowitz, J., Kaye, J., et al. 1986. Antigen Presenting Cell Lysis: Role in Immune Respones and Effect on Quantitative Analyses of Antigen Presentation to T Cells. In: **Antigen Presentation and Processing**. eds. Pernis, B. and Vogel , H.J. (Academic Press) in press.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by   has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

_____

_____

NAME AND ADDRESS   DATE